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<b>(21) International Application Number:</b> PCT/US96/05095 <b>(22) International Filing Date:</b> 12 April 1996 (12.04.96)  <b>(30) Priority Data:</b> 08/422,560 14 April 1995 (14.04.95) US 08/468,793 6 June 1995 (06.06.95) US 08/611,546 5 March 1996 (05.03.96) US  <b>(71) Applicant:</b> ARCH DEVELOPMENT CORPORATION [US/US]; 1101 East 58th Street, Chicago, IL 60637 (US). <b>(72) Inventors:</b> HASELKORN, Robert; 5834 S. Stony Island Avenue, Chicago, IL 60637 (US). GORNICK, Piotr; Apartment 1705, 5050 S. Lake Shore Drive, Chicago, IL 60615 (US). <b>(74) Agent:</b> KITCHELL, Barbara, S.; Arnold, White & Durkee, P.O. Box 4433, Houston, TX 77210 (US).		<b>(81) Designated States:</b> AL, AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>Without international search report and to be republished upon receipt of that report.</i>
<b>(54) Title:</b> ACETYL-CoA CARBOXYLASE COMPOSITIONS AND METHODS OF USE		
<b>(57) Abstract</b>  The present invention provides isolated and purified polynucleotides that encode plant and cyanobacterial polypeptides that participate in the carboxylation of acetyl-CoA. Isolated cyanobacterial and plant polypeptides that catalyze acetyl-CoA carboxylation are also provided. Processes for altering acetyl-CoA carboxylation, increasing herbicide resistance of plants and identifying herbicide resistant variants of acetyl-CoA carboxylase are also provided.		

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**DESCRIPTION****ACETYL-CoA CARBOXYLASE COMPOSITIONS AND METHODS OF USE****5 1. BACKGROUND OF THE INVENTION**

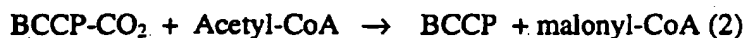
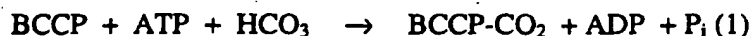
The present application is a continuation-in-part of U. S. Serial Number 08/422,560, filed April 14, 1995, which is a continuation-in-part of U. S. Serial Number 07/956,700, filed October 2, 1992; the entire texts and figures of which disclosures are specifically incorporated herein by reference without disclaimer. The United States government has certain rights in the present invention pursuant to Grant #90-34190-5207 from the United States Department of Agriculture.

**1.1 Field of the Invention**

The present invention relates to the field of molecular biology. More specifically, it concerns nucleic acid compositions comprising cyanobacterial and plant acetyl-CoA carboxylases (ACC), methods for making and using native and recombinant ACC polypeptides, and methods for making and using polynucleotides encoding ACC polypeptides.

**20 1.2 Description of the Related Art****1.2.1 Acetyl-CoA Carboxylase**

Acetyl-CoA carboxylase [ACCase; acetyl-CoA:carbon dioxide ligase (ADP-forming), EC 6.4.1.2] catalyzes the first committed step in *de novo* fatty acid biosynthesis, the addition of CO<sub>2</sub> to acetyl-CoA to yield malonyl-CoA. It belongs to a group of carboxylases that use biotin as cofactor and bicarbonate as a source of the carboxyl group. ACC catalyzes the addition of CO<sub>2</sub> to acetyl-CoA to yield malonyl-CoA in two steps as shown below.



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First, biotin becomes carboxylated at the expense of ATP. The carboxyl group is then transferred to Ac-CoA (Knowles, 1989). This irreversible reaction is the committed step in fatty acid synthesis and is a target for multiple regulatory mechanisms. Reaction (1) is catalyzed by biotin carboxylase (BC); reaction (2) by transcarboxylase (TC); BCCP = biotin carboxyl carrier protein.

There are two types of ACC: prokaryotic ACC in which the three functional domains: biotin carboxylase (BC), biotin carboxyl carrier protein (BCCP) and carboxyltransferase (CT) are located on separable subunits (e.g., *E. coli*, *P. aeruginosa*, *Anabaena*, *Synechococcus* and probably pea chloroplast) and eukaryotic ACC in which all the domains are located on one large polypeptide (e.g., rat, chicken, yeast, diatom and wheat).

*E. coli* ACC consists of a dimer of 49-kDa BC monomers, a dimer of 17-kDa BCCP monomers and a CT tetramer containing two each of 33-kDa and 35-kDa subunits. The primary structures of all of the *E. coli* ACC subunits (Alix, 1989; Muramatsu and Mizuno, 1989; Kondo *et al.*, 1991; Li and Cronan, 1992; Li and Cronan, 1992) as well as the structure of the BC and BCCP of *Anabaena* 7120 (Gornicki *et al.*, 1993), and *P. aeruginosa* (Best and Knauf, 1993) are known, based on the gene sequences. The genes encoding the subunits of *E. coli* ACC are called: *accA* (CT  $\alpha$  subunit), *accB* (BCCP), *accC* (BC) and *accD* (CT  $\beta$  subunit). *accC* and *accB* form one operon, while *accA* and *accD* are not linked to each other or to *accCB* (Li and Cronan, 1992). In cyanobacteria, *accC* and *accB* are unlinked as well (Gornicki *et al.*, 1993).

Yeast, rat, chicken and human ACCs are cytoplasmic enzymes consisting of 250- to 280-kDa subunits while diatom ACC is most likely a chloroplast enzyme consisting of 230-kDa subunits. Their primary structure has been deduced from cDNA sequences (Al-feel *et al.*, 1992; Lopez-Casillas *et al.*, 1988; Takai *et al.*, 1988; Roessler and Ohlrogge, 1993; Ha *et al.*, 1994). In eukaryotes, homologs of the four bacterial genes are fused in the following order: *accC*, *accB*, *accD* and *accA*. Animal ACC activity varies with the rate of fatty acid synthesis or energy requirements in different nutritional, hormonal and developmental states. In the rat, ACC mRNA is



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transcribed using different promoters in different tissues and can be regulated by alternative splicing. The rat enzyme activity is also allosterically regulated by a number of metabolites and by reversible phosphorylation (Ha *et al.*, 1994 and references therein). The expression of the yeast gene was shown to be coordinated  
5 with phospholipid metabolism (Chirala, 1992; Haslacher *et al.*, 1993).

Much less is known relating to plant ACC. Early attempts at characterization of plant ACC led to the suggestion that it consisted of low molecular weight subunits similar to those of bacteria (Harwood, 1988). More recent efforts indicate that at least one plant isozyme is composed of >200-kDa subunits, similar to the enzyme from  
10 other eukaryotes (Egin-Buhler and Ebel, 1983; Slabas and Hellyer, 1985; Gornicki and Haselkorn, 1993; Egli *et al.*, 1993; Betty *et al.*, 1992).

While strong evolutionary conservation exists among biotin carboxylases and biotin carboxylase domains of all biotin-dependent carboxylases, BCCP domains show very little conservation outside the conserved sequence E(A/V)MKM (lysine  
15 residue is biotinylated) (Knowles, 1989; Samols *et al.*, 1988). Although the three functional domains of the *E. coli* ACC are located on separate polypeptides, plant ACC is quite different, having all 3 domains on a single polypeptide.

At least one form of plant ACC is located in plastids, the primary site of fatty acid synthesis. The gene encoding it, however, must be nuclear because no  
20 corresponding sequence has been seen in the complete chloroplast DNA sequences of tobacco, liverwort or rice. The idea that in some plants plastid ACC consisted of several smaller subunits was revived by the discovery of an *accD* homolog in some chloroplast genomes (Li and Cronan, 1992). Indeed, it has been shown that the product of this gene in pea binds two other peptides, one of which is biotinylated. The  
25 complex may be a chloroplast isoform of ACC in pea and some other plants (Sasaki *et al.*, 1993).

It has been shown recently that plants have indeed more than one form of ACCase (reviewed in Sasaki *et al.*, 1995). The one located in plastids, the primary site of plant fatty acid synthesis, can be either a eukaryotic-type high molecular weight  
30 multi-functional enzyme (*e.g.*, in wheat and maize) or a prokaryotic-type

multi-subunit enzyme (*e.g.*, in pea, soybean, tobacco and Arabidopsis). The other plant ACCase, located in the cytoplasm, is of the eukaryotic type.

In Graminae, genes for both cytosolic and plastid eukaryotic-type ACCase are nuclear. No ACCase coding sequence can be found in the complete sequence of rice chloroplast DNA.

In other plants, subunits of ACCase other than the carboxyltransferase subunit encoded by a homolog of the *E. coli* *accD* gene, present in the chloroplast genome (Sasaki *et al.*, 1995; Li and Cronan, 1992), must be also encoded in the nuclear DNA.

Like the vast majority of plastid proteins, plastid ACCases are synthesized in the cytoplasm and then transported into the plastid. The amino acid sequence of the cytosolic and some subunits of the plastid ACCases from several plants have been deduced from genomic or cDNA sequences (Egli *et al.*, 1995; Li and Cronan, 1992; Gornicki *et al.*, 1994; Schulte *et al.*, 1994; Shorrosh *et al.*, 1994; Shorrosh *et al.*, 1995; Roesler *et al.*, 1994; Anderson *et al.*, 1995).

There is experimental evidence suggesting that, in plants, ACCase activity controls carbon flow through the fatty acid pathway and therefore may serve as an important regulation point of plant metabolism (Page *et al.*, 1994; Post-Beitenmiller *et al.*, 1992; Shintani and Ohlrogge, 1995).

The possibility of different ACC isoforms, one present in plastids and another in the cytoplasm, is now accepted. The rationale behind the search for a cytoplasmic ACC isoform is the requirement for malonyl-CoA in this cellular compartment, where it is used in fatty acid elongation and synthesis of secondary metabolites. Indeed, two isoforms were found in maize, both consisting of >200-kDa subunits but differing in size, herbicide sensitivity and immunological properties. The major form was found to be located in mesophyll chloroplasts. It is also the major ACC in the endosperm and in embryos (Egli *et al.*, 1993).

### 1.2.2 Cyanobacteria

Unlike monocot plants, members of the cyanobacteria are resistant to these herbicide families. Cyanobacteria are prokaryotes that carry out green plant

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photosynthesis, evolving O<sub>2</sub> in the light. They are believed to be the evolutionary ancestors of chloroplasts. Virtually nothing is known about fatty acid biosynthesis in cyanobacteria.

5 *Synechococcus* is a unicellular obligate phototroph with an efficient DNA transformation system. Replicating vectors based on endogenous plasmids are available, and selectable markers include resistance to kanamycin, chloramphenicol, streptomycin and the PSII inhibitors diuron and atrazine. Inactivation and/or deletion of *Synechococcus* genes by transformation with suitable cloned material interrupted by resistance cassettes is well known in the art. Genes may also be replaced by  
10 specifically mutated versions using selection for closely linked resistance cassettes.

*Anabaena* differentiates specialized cells for nitrogen fixation when the culture is deprived of a source of combined nitrogen. The differentiated cells have a unique glycolipid envelope containing C26 and C28 fatty acids (Murata and Nishida, 1987), whose synthesis must start with the reaction catalyzed by ACC. Therefore ACC must  
15 be developmentally regulated in *Anabaena*. Powerful systems of genetic analysis exist for *Anabaena* as well (Golden *et al.*, 1987).

That cyanobacteria and plants are evolutionarily-related make the former useful sources of cloned genes for the isolation of plant cDNAs. This method is well known to those of skill in the art. For example, the cloned gene for the enzyme  
20 phytoene desaturase, which functions in the synthesis of carotenoids, isolated from cyanobacteria was used as a probe to isolate the cDNA for that gene from tomato (Pecker *et al.*, 1992).

### 1.2.3 Herbicide Resistance

25 Although the mechanisms of inhibition and resistance are unknown (Lichtenthaler, 1990), it has been shown that aryloxyphenoxypropionates and cyclohexane-1,3-dione derivatives, powerful herbicides effective against monocot weeds, inhibit fatty acid biosynthesis in sensitive plants.

The aryloxyphenoxypropionate class comprises derivatives of  
30 aryloxyphenoxy-propionic acid such as diclofop, fenoxaprop, fluazifop, haloxyfop,

propaquizafop and quizalofop. Several derivatives of cyclohexane-1,3-dione are also important post-emergence herbicides which also selectively inhibit monocot plants. This group comprises such compounds as oxydim, cycloxydim, clethodim, sethoxydim, and tralkoxydim.

5        Recently it has been determined that ACC is the target enzyme for both of these classes of herbicide at least in monocots. Dicotyledonous plants, on the other hand, such as soybean rape, sunflower, tobacco, canola, bean, tomato, potato, lettuce, spinach, carrot, alfalfa and cotton are resistant to these compounds, as are other eukaryotes and prokaryotes.

10        Important grain crops, such as wheat, rice, maize, barley, rye, and oats, however, are monocotyledonous plants, and are therefore sensitive to these herbicides. Thus herbicides of the aryloxyphenoxypropionate and cyclohexane-1,3-dione groups are not useful in the agriculture of these important grain crops owing to the inactivation of monocot ACC by such chemicals.

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#### 1.2.4 Deficiencies in the Prior Art

The genetic transformation of important commercial monocotyledonous agriculture crops with DNA segments encoding herbicide-resistant ACC enzymes would be a revolution in the farming of such grains as wheat, rice, maize, barley, rye, and oats. Moreover the availability for modulating the herbicide resistance of plants through the alteration of ACC-encoding DNA segments and the polypeptides themselves would be highly desirable. Methods of identifying and assaying the levels of ACC activity in these plants would also be important in genetically engineering grain crops and the like with desirable herbicide-resistant qualities. Likewise the availability of DNA segments encoding dicotyledonous ACC and nucleic acid segments derived therefrom would provide a much-needed means of genetically altering the activity of ACC *in vivo* and *in vitro*.

25        What is lacking in the prior art, therefore, is the identification of DNA segments encoding plant and cyanobacterial ACC enzymes, and the development of methods and processes for their use in creation of modified, transgenic plants which

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have altered herbicide resistance. Moreover, novel methods providing transgenic plants using DNA segments encoding ACC polypeptides to modulate ACC activity, fatty acid biosynthesis in general, and oil content of plant cells in specific, are greatly needed to provide transformed plants altered in such activity. Methods for  
5 determining ACC activity *in vivo* and quantitating herbicide resistance in plants would also represent major improvements over the current state of the art.

## 2. SUMMARY OF THE INVENTION

The present invention seeks to overcome these and other inherent deficiencies  
10 in the prior art by providing compositions comprising novel ACC polypeptides from plant and cyanobacterial species. The invention also provides novel DNA segments encoding eukaryotic and prokaryotic ACCs, and methods and processes for their use in regulating the oil content of plant tissues, for conferring and modulating resistance to particular herbicides in a variety of plant species, and for altering the activity of  
15 ACC in plant cells *in vivo*. Also disclosed are methods for determining herbicide resistance and kits for identifying the presence of plant ACC polypeptides and DNA segments.

### 2.1 ACC Genes and Polynucleotides

20 The present invention provides polynucleotides and polypeptides relating to a whole or a portion of acetyl-CoA carboxylase (ACC) of cyanobacteria and plants as well as processes using those polynucleotides and polypeptides.

As used herein the term "polynucleotide" means a sequence of nucleotides connected by phosphodiester linkages. A polynucleotide of the present invention can  
25 comprise from about 2 to about several hundred thousand base pairs. Preferably, a polynucleotide comprises from about 5 to about 150,000 base pairs. Preferred lengths of particular polynucleotides are set forth hereinafter.

A polynucleotide of the present invention can be a deoxyribonucleic acid (DNA) molecule or a ribonucleic acid (RNA) molecule. Where a polynucleotide is a  
30 DNA molecule, that molecule can be a gene or a cDNA molecule. Nucleotide bases

are indicated herein by a single letter code: adenine (A), guanine (G), thymine (T), cytosine (C), and uracil (U).

In one embodiment, the present invention contemplates isolated and purified polynucleotides comprising DNA segments encoding polypeptides which have the ability to catalyze the carboxylation of a biotin carboxyl carrier protein of a cyanobacterium. Preferably, the cyanobacterium is *Anabaena* or *Synechococcus*. A preferred *Anabaena* is *Anabaena* 7120. A preferred *Synechococcus* is *Anacystis nidulans* R2 (*Synechococcus* sp. strain PCC 7942).

Preferably, a polypeptide is a biotin carboxylase enzyme of a cyanobacterium. This enzyme is a subunit of cyanobacterial acetyl-CoA carboxylase and participates in the carboxylation of acetyl-CoA. In a preferred embodiment, a BC polypeptide is encoded by a polynucleotide comprising an *accC* gene which has the nucleic acid sequence of SEQ ID NO:5 (*Anabaena accC*) or SEQ ID NO:7 (*Synechococcus accC*), or functional equivalents thereof. The BC polypeptide preferably comprises the amino acid sequence of SEQ ID NO:6 (*Anabaena* BC) or SEQ ID NO:8 (*Synechococcus* BC), or functional equivalents thereof.

In a second embodiment, the present invention contemplates isolated and purified polynucleotides comprising DNA segments encoding a biotin carboxyl carrier protein of a cyanobacterium. Preferably, the cyanobacterium is *Anabaena* or *Synechococcus*. A preferred *Anabaena* is *Anabaena* 7120. A preferred *Synechococcus* is *Anacystis nidulans* R2 (*Synechococcus* sp. strain PCC 7942).

Preferably, a polypeptide is a biotin carboxyl carrier protein of a cyanobacterium. This polypeptide is a subunit of cyanobacterial acetyl-CoA carboxylase and participates in the carboxylation of acetyl-CoA. In a preferred embodiment, a BCCP polypeptide is encoded by a polynucleotide comprising an *accB* gene which has the nucleic acid sequence of SEQ ID NO:1 (*Anabaena accB*) or SEQ ID NO:3 (*Synechococcus accB*), or functional equivalents thereof. The BCCP polypeptide preferably comprises the amino acid sequence of SEQ ID NO:2 (*Anabaena* BCCP) or SEQ ID NO:4 (*Synechococcus* BCCP), or functional equivalents thereof.

In a third embodiment, the present invention contemplates isolated and purified polynucleotides comprising DNA segments encoding a carboxyltransferase protein of a cyanobacterium. Preferably, the cyanobacterium is *Anabaena* or *Synechococcus*. A preferred *Anabaena* is *Anabaena* 7120. A preferred  
5 *Synechococcus* is *Anacystis nidulans* R2 (*Synechococcus* sp. strain PCC 7942).

Preferably, a polypeptide is a carboxyltransferase  $\alpha$  or  $\beta$  subunit protein of a cyanobacterium. These polypeptides are subunits of cyanobacterial acetyl-CoA carboxylase and participate in the carboxylation of acetyl-CoA. In a preferred embodiment, a CT $\alpha$  polypeptide is encoded by a polynucleotide comprising an *accA*  
10 gene which has the nucleic acid sequence of SEQ ID NO:11 (*Synechococcus accA*), or a functional equivalent thereof. The CT $\alpha$  polypeptide preferably comprises the amino acid sequence of SEQ ID NO:12 (*Synechococcus* CT $\alpha$ ), or a functional equivalent thereof.

In a fourth embodiment, the present invention contemplates isolated and  
15 purified polynucleotides comprising DNA segments encoding an acetyl-CoA carboxylase protein of a plant. Preferably, the plant is a monocotyledonous or a dicotyledonous plant. An exemplary and preferred monocotyledonous plant is wheat, rice, maize, barley, rye, oats or timothy grass. An exemplary and preferred dicotyledonous plant is soybean, rape, sunflower, tobacco, *Arabidopsis*, petunia, pea,  
20 canola, bean, tomato, potato, lettuce, spinach, alfalfa, cotton or carrot. A preferred monocotyledonous plant is wheat, and a preferred dicotyledonous plant is canola.

Preferably, a polypeptide is an acetyl-CoA carboxylase (ACC) protein of a plant. This polypeptide participates in the carboxylation of acetyl-CoA. In a preferred embodiment, an ACC polypeptide is encoded by a polynucleotide comprising an ACC  
25 cDNA which has the nucleic acid sequence of SEQ ID NO:9 (wheat ACC) or SEQ ID NO:19 (canola ACC), or functional equivalents thereof. The ACC polypeptide preferably comprises the amino acid sequence of SEQ ID NO:10 or SEQ ID NO:31 (wheat ACC) or SEQ ID NO:20 (canola ACC), or functional equivalents thereof.

In yet another aspect, the present invention provides an isolated and purified  
30 DNA molecule comprising a promoter operatively linked to a coding region that

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encodes (1) a polypeptide having the ability to catalyze the carboxylation of a biotin carboxyl carrier protein of a cyanobacterium, (2) a biotin carboxyl carrier protein of a cyanobacterium or (3) a plant polypeptide having the ability to catalyze the carboxylation of acetyl-CoA, which coding region is operatively linked to a transcription-terminating region, whereby said promoter drives the transcription of said coding region.

In another aspect, the present invention provides an isolated polypeptide having the ability to catalyze the carboxylation of a biotin carboxyl carrier protein of a cyanobacterium such as *Synechococcus*. Preferably a biotin carboxyl carrier protein gene includes the nucleic acid sequence of SEQ ID NO:2 and the polypeptide has the amino acid residue sequence of SEQ ID NO:6.

## 2.2 ACC Polypeptides and Anti-ACC Antibodies

The present invention also provides (1) an isolated and purified biotin carboxyl carrier protein of a cyanobacterium such as *Anabaena* or *Synechococcus*, which protein includes the amino acid residue sequence of SEQ ID NO:2 or SEQ ID NO:4, respectively; (2) an isolated and purified biotin carboxylase of a cyanobacterium such as *Anabaena* or *Synechococcus*, which protein includes the amino acid residue sequence of SEQ ID NO:6 or SEQ ID NO:8, respectively; (3) an isolated and purified carboxyltransferase  $\alpha$  subunit protein of a cyanobacterium such as *Synechococcus*, which protein includes the amino acid residue sequence of SEQ ID NO:12; (4) an isolated and purified monocotyledonous plant polypeptide from wheat having a molecular weight of about 220 kDa, dimers of which have the ability to catalyze the carboxylation of acetyl-CoA, which protein includes the amino acid sequence of SEQ ID NO:10 or SEQ ID NO:31; and (5) an isolated and purified dicotyledonous plant polypeptide from canola having the ability to catalyze the carboxylation of acetyl-CoA, which protein includes the amino acid sequence of SEQ ID NO:20.

Another aspect of the invention concerns methods and compositions for the use of the novel peptides of the invention in the production of anti-ACC antibodies.



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The present invention also provides methods for identifying ACC and ACC-related polypeptides, which methods comprise contacting a sample suspected of containing such polypeptides with an immunologically effective amount of a composition comprising one or more specific anti-ACC antibodies disclosed herein. Peptides that include the amino acid sequence of any of SEQ ID NO:4 through SEQ ID NO:8 and their derivatives will be preferred for use in generating such anti-ACC antibodies. Samples which may be tested or assayed for the presence of such ACC and ACC-related polypeptides include whole cells, cell extracts, cell homogenates, cell-free supernatants, and the like. Such cells may be either eukaryotic (such as plant cells) or prokaryotic (such as cyanobacterial and bacterial cells).

In certain aspects, diagnostic reagents comprising the novel peptides of the present invention and/or DNA segments which encode them have proven useful as test reagents for the detection of ACC and ACC-related polypeptides.

### 2.3 ACC Transformation and Identification of Herbicide-Resistant Variants

In yet another aspect, the present invention provides a process of modulating the herbicide resistance of a plant cell by a process of transforming the plant cell with a DNA molecule comprising a promoter operatively linked to a coding region that encodes a herbicide resistant polypeptide having the ability to catalyze the carboxylation of acetyl-CoA, which coding region is operatively linked to a transcription-terminating region, whereby the promoter is capable of driving the transcription of the coding region in a monocotyledonous plant.

Preferably, a polypeptide is an acetyl-CoA carboxylase enzyme and, more preferably, a plant acetyl-CoA carboxylase. In a preferred embodiment, a coding region includes the DNA sequence of SEQ ID NO:9 or SEQ ID NO:19 and a promoter is CaMV35.

In a preferred embodiment, a cell is a cyanobacterium or a plant cell and a plant polypeptide is a monocotyledonous plant acetyl-CoA carboxylase enzyme such as wheat acetyl-CoA carboxylase enzyme. The present invention also provides a transformed cyanobacterium produced in accordance with such a process.

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The present invention still further provides a process for determining the inheritance of plant resistance to herbicides of the aryloxyphenoxypropionate or cyclohexane-1,3-dione classes, which generally involves measuring resistance to these herbicides in a parental plant line and in the progeny of the parental plant line, detecting the presence of complexes between DNA restriction fragments and the ACC gene, and then correlating the herbicide resistance of the parental and progeny plants with the presence of particular sizes of ACC gene-containing DNA fragments as an indication of the inheritance of resistance to herbicides of these classes.

Preferably, the acetyl-CoA carboxylase is a dicotyledonous plant acetyl-CoA carboxylase enzyme or a mutated monocotyledonous plant acetyl-CoA carboxylase that confers herbicide resistance or a hybrid acetyl-CoA carboxylase comprising a portion of a dicotyledonous plant acetyl-CoA carboxylase, a portion of a monocotyledonous plant acetyl-CoA carboxylase or one or more domains of a cyanobacterial acetyl-CoA carboxylase.

Where a cyanobacterium is transformed with a plant ACC DNA molecule, that cyanobacterium can be used to identify herbicide resistant mutations in the gene encoding ACC. In accordance with such a use, the present invention provides a process for identifying herbicide resistant variants of a plant acetyl-CoA carboxylase comprising the steps of:

- (a) transforming cyanobacteria with a DNA molecule that encodes a monocotyledonous plant acetyl-CoA carboxylase enzyme to form transformed or transfected cyanobacteria;
- (b) inactivating cyanobacterial acetyl-CoA carboxylase;
- (c) exposing the transformed cyanobacteria to an effective herbicidal amount of a herbicide that inhibits acetyl-CoA carboxylase activity;
- (d) identifying transformed cyanobacteria that are resistant to the herbicide; and
- (e) characterizing DNA that encodes acetyl-CoA carboxylase from the cyanobacteria of step (d).

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Means for transforming cyanobacteria as well as expression vectors used for such transformation are preferably the same as set forth above. In a preferred embodiment, cyanobacteria are transformed or transfected with an expression vector comprising a coding region that encodes wheat ACC. Cyanobacteria resistant to the herbicide are identified. Identifying comprises growing or culturing transformed cells in the presence of the herbicide and recovering those cells that survive herbicide exposure. Transformed, herbicide-resistant cells are then grown in culture, collected and total DNA extracted using standard techniques. ACC DNA is isolated, amplified if needed and then characterized by comparing that DNA with DNA from ACC known to be inhibited by that herbicide.

In still yet another aspect, the present invention provides a process for identifying herbicide resistant variants of a plant acetyl-CoA carboxylase. Such methods generally involve transforming a cyanobacterium or a bacterium or a yeast cell with a DNA molecule that encodes a plant acetyl-CoA carboxylase enzyme, inactivating the host-cell acetyl-CoA carboxylase, and exposing the cells to a herbicide that inhibits monocotyledonous plant acetyl-CoA carboxylase activity. Transformed cells may be identified which are resistant to the herbicide; and the DNA that encodes resistant acetyl-CoA carboxylase in these transformed cells may be examined and characterized.

#### 2.4 ACC Transgenes and Transgenic Plants

In yet another aspect, the present invention provides a process of altering the carboxylation of acetyl-CoA in a cell comprising transforming the cell with a DNA molecule comprising a promoter operatively linked to a coding region that encodes a plant polypeptide having the ability to catalyze the carboxylation of acetyl-CoA, which coding region is operatively linked to a transcription-terminating region, whereby the promoter is capable of driving the transcription of the coding region in the cell. The invention also provides a means of reducing the amount of ACC in plants by expression of ACC antisense mRNA.

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Another aspect of the invention relates generally to transgenic plants which express genes or gene segments encoding the novel polypeptide compositions disclosed herein. As used herein, the term "transgenic plants" is intended to refer to plants that have incorporated DNA sequences, including but not limited to genes which are perhaps not normally present, DNA sequences not normally transcribed into RNA or translated into a protein ("expressed"), or any other genes or DNA sequences which one desires to introduce into the non-transformed plant, such as genes which may normally be present in the non-transformed plant but which one desires to either genetically engineer or to have altered expression. It is contemplated that in some instances the genome of transgenic plants of the present invention will have been augmented through the stable introduction of the transgene. However, in other instances, the introduced gene will replace an endogenous sequence.

A preferred gene which may be introduced includes, for example, the ACC DNA sequences from cyanobacterial or plant origin, particularly those described herein which are obtained from the cyanobacterial species *Synechococcus* or *Anabaena*, or from plant species such as wheat or canola, of any of those sequences which have been genetically engineered to decrease or increase the activity of the ACC in such transgenic species.

Vectors, plasmids, cosmids, YACs (yeast artificial chromosomes) and DNA segments for use in transforming such cells will, of course, generally comprise either the cDNA, gene or gene sequences of the present invention, and particularly those encoding ACC. These DNA constructs can further include structures such as promoters, enhancers, polylinkers, or even regulatory genes as desired. The DNA segment or gene may encode either a native or modified ACC, which will be expressed in the resultant recombinant cells, and/or which will impart an improved phenotype to the regenerated plant.

Such transgenic plants may be desirable for increasing the herbicide resistance of a monocotyledonous plant, by incorporating into such a plant, a transgenic DNA segment encoding a plant acetyl-CoA carboxylase enzyme which is resistant to herbicide inactivation, e.g., a dicotyledonous ACC gene. Alternatively a

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cyanobacterial ACC polypeptide-encoding DNA segment could also be used to prepare a transgenic plant with increased resistance to herbicide inactivation.

Alternatively transgenic plants may be desirable having an decreased herbicide resistance. This would be particularly desirable in creating transgenic plants which are more sensitive to such herbicides. Such a herbicide-sensitive plant could be prepared by incorporating into such a plant, a transgenic DNA segment encoding a plant acetyl-CoA carboxylase enzyme which is sensitive to herbicide inactivation, *e.g.*, a monocotyledonous ACC gene, or a mutated dicotyledonous or cyanobacterial ACC-encoding gene.

In other aspects of the present invention, the invention concerns processes of modifying the oil content of a plant cell. Such modifications generally involve expressing in such plant cells transgenic DNA segments encoding a plant or cyanobacterial acetyl-CoA carboxylase composition of the present invention. Such processes would generally result in increased expression of ACC and hence, increased oil production in such cells. Alternatively, when it is desirable to decrease the oil production of such cells, ACC-encoding transgenic DNA segments or antisense (complementary) DNA segments to genomic ACC-encoding DNA sequences may be used to transform cells.

Either process may be facilitated by introducing into such cells DNA segments encoding a plant or cyanobacterial acetyl-CoA carboxylase polypeptide, as long as the resulting transgenic plant expresses the acetyl-CoA carboxylase-encoding transgene.

The present invention also provides a transformed plant produced in accordance with the above process as well as a transgenic plant and a transgenic plant seed having incorporated into its genome a transgene that encodes a herbicide resistant polypeptide having the ability to catalyze the carboxylation of acetyl-CoA. All such transgenic plants having incorporated into their genome transgenic DNA segments encoding plant or cyanobacterial acetyl-CoA carboxylase polypeptides are aspects of this invention.

## 2.5 ACC Screening and Immunodetection Kits

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The present invention contemplates methods and kits for screening samples suspected of containing ACC polypeptides or ACC-related polypeptides, or cells producing such polypeptides. Said kit can contain a nucleic acid segment or an antibody of the present invention. The kit can contain reagents for detecting an interaction between a sample and a nucleic acid or antibody of the present invention. 5 The provided reagent can be radio-, fluorescently- or enzymatically-labeled. The kit can contain a known radiolabeled agent capable of binding or interacting with a nucleic acid or antibody of the present invention.

The reagent of the kit can be provided as a liquid solution, attached to a solid support or as a dried powder. Preferably, when the reagent is provided in a liquid solution, the liquid solution is an aqueous solution. Preferably, when the reagent provided is attached to a solid support, the solid support can be chromatograph media, a test plate having a plurality of wells, or a microscope slide. When the reagent provided is a dry powder, the powder can be reconstituted by the addition of a suitable solvent, that may be provided. 10 15

In still further embodiments, the present invention concerns immunodetection methods and associated kits. It is proposed that the ACC peptides of the present invention may be employed to detect antibodies having reactivity therewith, or, alternatively, antibodies prepared in accordance with the present invention, may be employed to detect ACC or ACC-related epitope-containing peptides. In general, these methods will include first obtaining a sample suspected of containing such a protein, peptide or antibody, contacting the sample with an antibody or peptide in accordance with the present invention, as the case may be, under conditions effective to allow the formation of an immunocomplex, and then detecting the presence of the immunocomplex. 20 25

In general, the detection of immunocomplex formation is quite well known in the art and may be achieved through the application of numerous approaches. For example, the present invention contemplates the application of ELISA, RIA, immunoblot (*e.g.*, dot blot), indirect immunofluorescence techniques and the like. 30 Generally, immunocomplex formation will be detected through the use of a label,

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such as a radiolabel or an enzyme tag (such as alkaline phosphatase, horseradish peroxidase, or the like). Of course, one may find additional advantages through the use of a secondary binding ligand such as a second antibody or a biotin/avidin ligand binding arrangement, as is known in the art.

5           For assaying purposes, it is proposed that virtually any sample suspected of comprising either an ACC peptide or an ACC-related peptide or antibody sought to be detected, as the case may be, may be employed. It is contemplated that such embodiments may have application in the titering of antigen or antibody samples, in the selection of hybridomas, and the like. In related embodiments, the present  
10          invention contemplates the preparation of kits that may be employed to detect the presence of ACC or ACC-related proteins or peptides and/or antibodies in a sample. Samples may include cells, cell supernatants, cell suspensions, cell extracts, enzyme fractions, protein extracts, or other cell-free compositions suspected of containing ACC peptides. Generally speaking, kits in accordance with the present invention will  
15          include a suitable ACC peptide or an antibody directed against such a protein or peptide, together with an immunodetection reagent and a means for containing the antibody or antigen and reagent. The immunodetection reagent will typically comprise a label associated with the antibody or antigen, or associated with a secondary binding ligand. Exemplary ligands might include a secondary antibody  
20          directed against the first antibody or antigen or a biotin or avidin (or streptavidin) ligand having an associated label. Of course, as noted above, a number of exemplary labels are known in the art and all such labels may be employed in connection with the present invention.

          The container will generally include a vial into which the antibody, antigen or  
25          detection reagent may be placed, and preferably suitably aliquotted. The kits of the present invention will also typically include a means for containing the antibody, antigen, and reagent containers in close confinement for commercial sale. Such containers may include injection or blow-molded plastic containers into which the desired vials are retained.

## 2.6 ELISAs and Immunoprecipitation

ELISAs may be used in conjunction with the invention. In an ELISA assay, proteins or peptides incorporating ACC antigen sequences are immobilized onto a selected surface, preferably a surface exhibiting a protein affinity such as the wells of a polystyrene microtiter plate. After washing to remove incompletely adsorbed material, it is desirable to bind or coat the assay plate wells with a nonspecific protein that is known to be antigenically neutral with regard to the test antisera such as bovine serum albumin (BSA), casein or solutions of milk powder. This allows for blocking of nonspecific adsorption sites on the immobilizing surface and thus reduces the background caused by nonspecific binding of antisera onto the surface.

After binding of antigenic material to the well, coating with a non-reactive material to reduce background, and washing to remove unbound material, the immobilizing surface is contacted with the antisera or clinical or biological extract to be tested in a manner conducive to immune complex (antigen/antibody) formation. Such conditions preferably include diluting the antisera with diluents such as BSA, bovine gamma globulin (BGG) and phosphate buffered saline (PBS)/Tween®. These added agents also tend to assist in the reduction of nonspecific background. The layered antisera is then allowed to incubate for from about 2 to about 4 hours, at temperatures preferably on the order of about 25° to about 27°C. Following incubation, the antisera-contacted surface is washed so as to remove non-immunocomplexed material. A preferred washing procedure includes washing with a solution such as PBS/Tween®, or borate buffer.

Following formation of specific immunocomplexes between the test sample and the bound antigen, and subsequent washing, the occurrence and even amount of immunocomplex formation may be determined by subjecting same to a second antibody having specificity for the first. To provide a detecting means, the second antibody will preferably have an associated enzyme that will generate a color development upon incubating with an appropriate chromogenic substrate. Thus, for example, one will desire to contact and incubate the antisera-bound surface with a urease or peroxidase-conjugated anti-human IgG for a period of time and under



conditions which favor the development of immunocomplex formation (*e.g.*, incubation for 2 hours at room temperature in a PBS-containing solution such as PBS Tween®).

5 After incubation with the second enzyme-tagged antibody, and subsequent to washing to remove unbound material, the amount of label is quantified by incubation with a chromogenic substrate such as urea and bromocresol purple or 2,2'-azino-di-(3-ethyl-benzthiazoline)-6-sulfonic acid (ABTS) and H<sub>2</sub>O<sub>2</sub>, in the case of peroxidase as the enzyme label. Quantification is then achieved by measuring the degree of color generation, *e.g.*, using a visible spectra spectrophotometer.

10 The antibodies of the present invention are particularly useful for the isolation of antigens by immunoprecipitation. Immunoprecipitation involves the separation of the target antigen component from a complex mixture, and is used to discriminate or isolate minute amounts of protein. For the isolation of membrane proteins cells must be solubilized into detergent micelles. Nonionic salts are preferred, since other agents  
15 such as bile salts, precipitate at acid pH or in the presence of bivalent cations.

In an alternative embodiment the antibodies of the present invention are useful for the close juxtaposition of two antigens. This is particularly useful for increasing the localized concentration of antigens, *e.g.* enzyme-substrate pairs.

## 20 2.7 Western Blots

The compositions of the present invention will find great use in immunoblot or western blot analysis. The anti-peptide antibodies may be used as high-affinity primary reagents for the identification of proteins immobilized onto a solid support matrix, such as nitrocellulose, nylon or combinations thereof. In conjunction with  
25 immunoprecipitation, followed by gel electrophoresis, these may be used as a single step reagent for use in detecting antigens against which secondary reagents used in the detection of the antigen cause an adverse background. This is especially useful when the antigens studied are immunoglobulins (precluding the use of immunoglobulins binding bacterial cell wall components), the antigens studied cross-react with the

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detecting agent, or they migrate at the same relative molecular weight as a cross-reacting signal.

Immunologically-based detection methods for use in conjunction with Western blotting include enzymatically-, radiolabel-, or fluorescently-tagged secondary  
5 antibodies against the toxin moiety are considered to be of particular use in this regard.

## 2.8 Epitopic Core Sequences

The present invention is also directed to protein or peptide compositions, free  
10 from total cells and other peptides, which comprise a purified protein or peptide which incorporates an epitope that is immunologically cross-reactive with one or more anti-ACC antibodies.

As used herein, the term "incorporating an epitope(s) that is immunologically cross-reactive with one or more anti-ACC antibodies" is intended to refer to a peptide  
15 or protein antigen which includes a primary, secondary or tertiary structure similar to an epitope located within an ACC polypeptide. The level of similarity will generally be to such a degree that monoclonal or polyclonal antibodies directed against the ACC polypeptide will also bind to, react with, or otherwise recognize, the cross-reactive peptide or protein antigen. Various immunoassay methods may be employed in  
20 conjunction with such antibodies, such as, for example, Western blotting, ELISA, RIA, and the like, all of which are known to those of skill in the art.

The identification of ACC immunodominant epitopes, and/or their functional equivalents, suitable for use in vaccines is a relatively straightforward matter. For example, one may employ the methods of Hopp, as taught in U.S. Patent 4,554,101,  
25 incorporated herein by reference, which teaches the identification and preparation of epitopes from amino acid sequences on the basis of hydrophilicity. The methods described in several other papers, and software programs based thereon, can also be used to identify epitopic core sequences (see, for example, Jameson and Wolf, 1988; Wolf *et al.*, 1988; U.S. Patent Number 4,554,101). The amino acid sequence of these

"epitopic core sequences" may then be readily incorporated into peptides, either through the application of peptide synthesis or recombinant technology.

Preferred peptides for use in accordance with the present invention will generally be on the order of 8 to 20 amino acids in length, and more preferably about 8 to about 15 amino acids in length. It is proposed that shorter antigenic ACC-derived peptides will provide advantages in certain circumstances, for example, in the preparation of vaccines or in immunologic detection assays. Exemplary advantages include the ease of preparation and purification, the relatively low cost and improved reproducibility of production, and advantageous biodistribution.

It is proposed that particular advantages of the present invention may be realized through the preparation of synthetic peptides which include modified and/or extended epitopic/immunogenic core sequences which result in a "universal" epitopic peptide directed to ACC and ACC-related sequences. These epitopic core sequences are identified herein in particular aspects as hydrophilic regions of the ACC polypeptide antigen. It is proposed that these regions represent those which are most likely to promote T-cell or B-cell stimulation, and, hence, elicit specific antibody production.

An epitopic core sequence, as used herein, is a relatively short stretch of amino acids that is "complementary" to, and therefore will bind, antigen binding sites on transferrin-binding protein antibodies. Additionally or alternatively, an epitopic core sequence is one that will elicit antibodies that are cross-reactive with antibodies directed against the peptide compositions of the present invention. It will be understood that in the context of the present disclosure, the term "complementary" refers to amino acids or peptides that exhibit an attractive force towards each other. Thus, certain epitope core sequences of the present invention may be operationally defined in terms of their ability to compete with or perhaps displace the binding of the desired protein antigen with the corresponding protein-directed antisera.

In general, the size of the polypeptide antigen is not believed to be particularly crucial, so long as it is at least large enough to carry the identified core sequence or sequences. The smallest useful core sequence anticipated by the present disclosure

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would generally be on the order of about 8 amino acids in length, with sequences on the order of 10 to 20 being more preferred. Thus, this size will generally correspond to the smallest peptide antigens prepared in accordance with the invention. However, the size of the antigen may be larger where desired, so long as it contains a basic epitopic core sequence.

The identification of epitopic core sequences is known to those of skill in the art, for example, as described in U.S. Patent 4,554,101, incorporated herein by reference, which teaches the identification and preparation of epitopes from amino acid sequences on the basis of hydrophilicity. Moreover, numerous computer programs are available for use in predicting antigenic portions of proteins (see *e.g.*, Jameson and Wolf, 1988; Wolf *et al.*, 1988). Computerized peptide sequence analysis programs (*e.g.*, DNASTar® software, DNASTar, Inc., Madison, WI) may also be useful in designing synthetic peptides in accordance with the present disclosure.

Syntheses of epitopic sequences, or peptides which include an antigenic epitope within their sequence, are readily achieved using conventional synthetic techniques such as the solid phase method (*e.g.*, through the use of commercially available peptide synthesizer such as an Applied Biosystems Model 430A Peptide Synthesizer). Peptide antigens synthesized in this manner may then be aliquotted in predetermined amounts and stored in conventional manners, such as in aqueous solutions or, even more preferably, in a powder or lyophilized state pending use.

In general, due to the relative stability of peptides, they may be readily stored in aqueous solutions for fairly long periods of time if desired, *e.g.*, up to six months or more, in virtually any aqueous solution without appreciable degradation or loss of antigenic activity. However, where extended aqueous storage is contemplated it will generally be desirable to include agents including buffers such as Tris or phosphate buffers to maintain a pH of about 7.0 to about 7.5. Moreover, it may be desirable to include agents which will inhibit microbial growth, such as sodium azide or Merthiolate. For extended storage in an aqueous state it will be desirable to store the solutions at 4°C, or more preferably, frozen. Of course, where the peptides are stored in a lyophilized or powdered state, they may be stored virtually indefinitely, *e.g.*, in

metered aliquots that may be rehydrated with a predetermined amount of water (preferably distilled) or buffer prior to use.

## 2.9 DNA Segments

5       The present invention also concerns DNA segments, that can be isolated from virtually any source, that are free from total genomic DNA and that encode the novel peptides disclosed herein. DNA segments encoding these peptide species may prove to encode proteins, polypeptides, subunits, functional domains, and the like of ACC-related or other non-related gene products. In addition these DNA segments may be  
10 synthesized entirely *in vitro* using methods that are well-known to those of skill in the art.

As used herein, the term "DNA segment" refers to a DNA molecule that has been isolated free of total genomic DNA of a particular species. Therefore, a DNA segment encoding an ACC peptide refers to a DNA segment that contains ACC  
15 coding sequences yet is isolated away from, or purified free from, total genomic DNA of the species from which the DNA segment is obtained. Included within the term "DNA segment", are DNA segments and smaller fragments of such segments, and also recombinant vectors, including, for example, plasmids, cosmids, phagemids, phage, viruses, and the like.

20       Similarly, a DNA segment comprising an isolated or purified ACC gene refers to a DNA segment which may include in addition to peptide encoding sequences, certain other elements such as, regulatory sequences, isolated substantially away from other naturally occurring genes or protein-encoding sequences. In this respect, the term "gene" is used for simplicity to refer to a functional protein-, polypeptide- or  
25 peptide-encoding unit. As will be understood by those in the art, this functional term includes both genomic sequences, cDNA sequences and smaller engineered gene segments that express, or may be adapted to express, proteins, polypeptides or peptides.

"Isolated substantially away from other coding sequences" means that the gene  
30 of interest, in this case, a gene encoding ACC, forms the significant part of the coding

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region of the DNA segment, and that the DNA segment does not contain large portions of naturally-occurring coding DNA, such as large chromosomal fragments or other functional genes or cDNA coding regions. Of course, this refers to the DNA segment as originally isolated, and does not exclude genes or coding regions later  
5 added to the segment by the hand of man.

In particular embodiments, the invention concerns isolated DNA segments and recombinant vectors incorporating DNA sequences that encode an ACC peptide species that includes within its amino acid sequence an amino acid sequence essentially as set forth in any of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID  
10 NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:20, and SEQ ID NO:31.

The term "a sequence essentially as set forth in any of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:20 and SEQ ID NO:31" means that the sequence substantially corresponds to a portion of the sequence of either SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8,  
15 SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:20 or SEQ ID NO:31, and has relatively few amino acids that are not identical to, or a biologically functional equivalent of, the amino acids of any of these sequences. The term "biologically functional equivalent" is well understood in the art and is further defined in detail herein (for example, see Preferred Embodiments). Accordingly, sequences that have between about 70% and  
20 about 80%, or more preferably between about 81% and about 90%, or even more preferably between about 91% and about 99% amino acid sequence identity or functional equivalence to the amino acids of any of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:20, and SEQ ID NO:31 will be sequences that are "essentially as set forth in any of SEQ ID  
25 NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:20, and SEQ ID NO:31."

It will also be understood that amino acid and nucleic acid sequences may include additional residues, such as additional N- or C-terminal amino acids or 5' or 3' sequences, and yet still be essentially as set forth in one of the sequences disclosed  
30 herein, so long as the sequence meets the criteria set forth above, including the

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5 maintenance of biological protein activity where protein expression is concerned. The addition of terminal sequences particularly applies to nucleic acid sequences that may, for example, include various non-coding sequences flanking either of the 5' or 3' portions of the coding region or may include various internal sequences, *i.e.*, introns, which are known to occur within genes.

10 The nucleic acid segments of the present invention, regardless of the length of the coding sequence itself, may be combined with other DNA sequences, such as promoters, polyadenylation signals, additional restriction enzyme sites, multiple cloning sites, other coding segments, and the like, such that their overall length may vary considerably. It is therefore contemplated that a nucleic acid fragment of almost any length may be employed, with the total length preferably being limited by the ease of preparation and use in the intended recombinant DNA protocol. For example, nucleic acid fragments may be prepared that include a short contiguous stretch encoding either of the peptide sequences disclosed in any of SEQ ID NO:2, SEQ ID  
15 NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:20 and SEQ ID NO:31, or that are identical to or complementary to DNA sequences which encode any of the peptides disclosed in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:20, and SEQ ID NO:31, and particularly those DNA segments disclosed in SEQ ID NO:1, SEQ ID  
20 NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:19, or SEQ ID NO:30. For example, DNA sequences such as about 14 nucleotides, and that are up to about 13,000, about 5,000, about 3,000, about 2,000, about 1,000, about 500, about 200, about 100, about 50, and about 14 base pairs in length (including all intermediate lengths) are also contemplated to be useful.

25 It will be readily understood that "intermediate lengths", in these contexts, means any length between the quoted ranges, such as 14, 15, 16, 17, 18, 19, 20, *etc.*; 21, 22, 23, *etc.*; 30, 31, 32, *etc.*; 50, 51, 52, 53, *etc.*; 100, 101, 102, 103, *etc.*; 150, 151, 152, 153, *etc.*; including all integers through the 200-500; 500-1,000; 1,000-2,000; 2,000-3,000; 3,000-5,000; 5,000-10,000, 10,000-12,000, 12,000-13,000 and up to and

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including sequences of about 13,000, 13,001, 13,002, or 13,003 nucleotides *etc.* and the like.

It will also be understood that this invention is not limited to the particular nucleic acid sequences which encode peptides of the present invention, or which  
5 encode the amino acid sequences of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:20, and SEQ ID NO:31, including those DNA sequences which are particularly disclosed in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID  
10 NO:19, and SEQ ID NO:30. Recombinant vectors and isolated DNA segments may therefore variously include the peptide-coding regions themselves, coding regions bearing selected alterations or modifications in the basic coding region, or they may encode larger polypeptides that nevertheless include these peptide-coding regions or may encode biologically functional equivalent proteins or peptides that have variant amino acids sequences.

15 The DNA segments of the present invention encompass biologically-functional equivalent peptides. Such sequences may arise as a consequence of codon redundancy and functional equivalency that are known to occur naturally within nucleic acid sequences and the proteins thus encoded. Alternatively, functionally-equivalent proteins or peptides may be created via the application of recombinant  
20 DNA technology, in which changes in the protein structure may be engineered, based on considerations of the properties of the amino acids being exchanged. Changes designed by man may be introduced through the application of site-directed mutagenesis techniques, *e.g.*, to introduce improvements to the antigenicity of the protein or to test mutants in order to examine activity at the molecular level.

25 If desired, one may also prepare fusion proteins and peptides, *e.g.*, where the peptide-coding regions are aligned within the same expression unit with other proteins or peptides having desired functions, such as for purification or immunodetection purposes (*e.g.*, proteins that may be purified by affinity chromatography and enzyme label coding regions, respectively).



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Recombinant vectors form further aspects of the present invention. Particularly useful vectors are contemplated to be those vectors in which the coding portion of the DNA segment, whether encoding a full length protein or smaller peptide, is positioned under the control of a promoter. The promoter may be in the form of the promoter that is naturally associated with a gene encoding peptides of the present invention, as may be obtained by isolating the 5' non-coding sequences located upstream of the coding segment or exon, for example, using recombinant cloning and/or PCR™ technology, in connection with the compositions disclosed herein.

10 In other embodiments, it is contemplated that certain advantages will be gained by positioning the coding DNA segment under the control of a recombinant, or heterologous, promoter. As used herein, a recombinant or heterologous promoter is intended to refer to a promoter that is not normally associated with a DNA segment encoding an ACC peptide in its natural environment. Such promoters may include  
15 promoters normally associated with other genes, and/or promoters isolated from any bacterial, viral, eukaryotic, or plant cell. Naturally, it will be important to employ a promoter that effectively directs the expression of the DNA segment in the cell type, organism, or even animal, chosen for expression. The use of promoter and cell type combinations for protein expression is generally known to those of skill in the art of  
20 molecular biology, for example, see Sambrook *et al.*, 1989. The promoters employed may be constitutive, or inducible, and can be used under the appropriate conditions to direct high level expression of the introduced DNA segment, such as is advantageous in the large-scale production of recombinant proteins or peptides. Appropriate promoter systems contemplated for use in high-level expression include, but are not  
25 limited to, the *Pichia* expression vector system (Pharmacia LKB Biotechnology).

In connection with expression embodiments to prepare recombinant proteins and peptides, it is contemplated that longer DNA segments will most often be used, with DNA segments encoding the entire peptide sequence being most preferred. However, it will be appreciated that the use of shorter DNA segments to direct the  
30 expression of ACC peptides or epitopic core regions, such as may be used to generate

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anti-ACC antibodies, also falls within the scope of the invention. DNA segments that encode peptide antigens from about 8 to about 50 amino acids in length, or more preferably, from about 8 to about 30 amino acids in length, or even more preferably, from about 8 to about 20 amino acids in length are contemplated to be particularly  
5 useful. Such peptide epitopes may be amino acid sequences which comprise contiguous amino acid sequences from any of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:20, or SEQ ID NO:31.

In addition to their use in directing the expression of ACC peptides of the  
10 present invention, the nucleic acid sequences contemplated herein also have a variety of other uses. For example, they also have utility as probes or primers in nucleic acid hybridization embodiments. As such, it is contemplated that nucleic acid segments that comprise a sequence region that consists of at least a 14 nucleotide long contiguous sequence that has the same sequence as, or is complementary to, a 14  
15 nucleotide long contiguous DNA segment any of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:19, and SEQ ID NO:30 will find particular utility. Longer contiguous identical or complementary sequences, *e.g.*, those of about 20, 30, 40, 50, 100, 200, 500, 1,000, 2,000, 5,000, 8,000, 10,000, 12,000, 13,000 *etc.* (including all intermediate lengths and up to and  
20 including full-length sequences will also be of use in certain embodiments.

The ability of such nucleic acid probes to specifically hybridize to ACC-encoding sequences will enable them to be of use in detecting the presence of complementary sequences in a given sample. However, other uses are envisioned, including the use of the sequence information for the preparation of mutant species  
25 primers, or primers for use in preparing other genetic constructions.

Nucleic acid molecules having sequence regions consisting of contiguous nucleotide stretches of 10-14, 15-20, 30, 50, or even of 100-200 nucleotides or so, identical or complementary to DNA sequences of any of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:19,  
30 and SEQ ID NO:30 are particularly contemplated as hybridization probes for use in,

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*e.g.*, Southern and Northern blotting. Smaller fragments will generally find use in hybridization embodiments, wherein the length of the contiguous complementary region may be varied, such as between about 10-14 and about 100 or 200 nucleotides, but larger contiguous complementarity stretches may be used, according to the length  
5 complementary sequences one wishes to detect.

The use of a hybridization probe of about 14 nucleotides in length allows the formation of a duplex molecule that is both stable and selective. Molecules having contiguous complementary sequences over stretches greater than 14 bases in length are generally preferred, though, in order to increase stability and selectivity of the  
10 hybrid, and thereby improve the quality and degree of specific hybrid molecules obtained. One will generally prefer to design nucleic acid molecules having gene-complementary stretches of 15 to 20 contiguous nucleotides, or even longer where desired.

Of course, fragments may also be obtained by other techniques such as, *e.g.*,  
15 by mechanical shearing or by restriction enzyme digestion. Small nucleic acid segments or fragments may be readily prepared by, for example, directly synthesizing the fragment by chemical means, as is commonly practiced using an automated oligonucleotide synthesizer. Also, fragments may be obtained by application of nucleic acid reproduction technology, such as the PCR™ technology of U.S. Patents  
20 4,683,195 and 4,683,202 (each incorporated herein by reference), by introducing selected sequences into recombinant vectors for recombinant production, and by other recombinant DNA techniques generally known to those of skill in the art of molecular biology.

Accordingly, the nucleotide sequences of the invention may be used for their  
25 ability to selectively form duplex molecules with complementary stretches of DNA fragments. Depending on the application envisioned, one will desire to employ varying conditions of hybridization to achieve varying degrees of selectivity of probe towards target sequence. For applications requiring high selectivity, one will typically desire to employ relatively stringent conditions to form the hybrids, *e.g.*, one will  
30 select relatively low salt and/or high temperature conditions, such as provided by

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about 0.02 M to about 0.15 M NaCl at temperatures of about 50°C to about 70°C. Such selective conditions tolerate little, if any, mismatch between the probe and the template or target strand, and would be particularly suitable for isolating ACC-encoding DNA segments. Detection of DNA segments via hybridization is well-known to those of skill in the art, and the teachings of U.S. Patents 4,965,188 and 5,176,995 (each incorporated herein by reference) are exemplary of the methods of hybridization analyses. Teachings such as those found in the texts of Maloy *et al.*, 1993; Segal 1976; Proskop, 1991; and Kuby, 1991, are particularly relevant.

Of course, for some applications, for example, where one desires to prepare mutants employing a mutant primer strand hybridized to an underlying template or where one seeks to isolate ACC-encoding sequences from related species, functional equivalents, or the like, less stringent hybridization conditions will typically be needed in order to allow formation of the heteroduplex. In these circumstances, one may desire to employ conditions such as about 0.15 M to about 0.9 M salt, at temperatures ranging from about 20°C to about 55°C. Cross-hybridizing species can thereby be readily identified as positively hybridizing signals with respect to control hybridizations. In any case, it is generally appreciated that conditions can be rendered more stringent by the addition of increasing amounts of formamide, which serves to destabilize the hybrid duplex in the same manner as increased temperature. Thus, hybridization conditions can be readily manipulated, and thus will generally be a method of choice depending on the desired results.

In certain embodiments, it will be advantageous to employ nucleic acid sequences of the present invention in combination with an appropriate means, such as a label, for determining hybridization. A wide variety of appropriate indicator means are known in the art, including fluorescent, radioactive, enzymatic or other ligands, such as avidin/biotin, which are capable of giving a detectable signal. In preferred embodiments, one will likely desire to employ a fluorescent label or an enzyme tag, such as urease, alkaline phosphatase or peroxidase, instead of radioactive or other environmental undesirable reagents. In the case of enzyme tags, colorimetric indicator substrates are known that can be employed to provide a means visible to the human

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eye or spectrophotometrically, to identify specific hybridization with complementary nucleic acid-containing samples.

In general, it is envisioned that the hybridization probes described herein will be useful both as reagents in solution hybridization as well as in embodiments employing a solid phase. In embodiments involving a solid phase, the test DNA (or RNA) is adsorbed or otherwise affixed to a selected matrix or surface. This fixed, single-stranded nucleic acid is then subjected to specific hybridization with selected probes under desired conditions. The selected conditions will depend on the particular circumstances based on the particular criteria required (depending, for example, on the G+C content, type of target nucleic acid, source of nucleic acid, size of hybridization probe, *etc.*). Following washing of the hybridized surface so as to remove nonspecifically bound probe molecules, specific hybridization is detected, or even quantitated, by means of the label.

## 2.10 Biological Functional Equivalents

Modification and changes may be made in the structure of the peptides of the present invention and DNA segments which encode them and still obtain a functional molecule that encodes a protein or peptide with desirable characteristics. The following is a discussion based upon changing the amino acids of a protein to create an equivalent, or even an improved, second-generation molecule. The amino acid changes may be achieved by changing the codons of the DNA sequence, according to the codons listed in Table 1.

TABLE 1

Amino Acids		Codons				
Alanine	Ala	A	GCA	GCC	GCG	GCU
Cysteine	Cys	C	UGC	UGU		
Aspartic acid	Asp	D	GAC	GAU		
Glutamic acid	Glu	E	GAA	GAG		
Phenylalanine	Phe	F	UUC	UUU		

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Amino Acids		Codons							
Glycine	Gly	G	GGA	GGC	GGG	GGU			
Histidine	His	H	CAC	CAU					
Isoleucine	Ile	I	AUA	AUC	AUU				
Lysine	Lys	K	AAA	AAG					
Leucine	Leu	L	UUA	UUG	CUA	CUC	CUG	CUU	
Methionine	Met	M	AUG						
Asparagine	Asn	N	AAC	AAU					
Proline	Pro	P	CCA	CCC	CCG	CCU			
Glutamine	Gln	Q	CAA	CAG					
Arginine	Arg	R	AGA	AGG	CGA	CGC	CGG	CGU	
Serine	Ser	S	AGC	AGU	UCA	UCC	UCG	UCU	
Threonine	Thr	T	ACA	ACC	ACG	ACU			
Valine	Val	V	GUA	GUC	GUG	GUU			
Tryptophan	Trp	W	UGG						
Tyrosine	Tyr	Y	UAC	UAU					

For example, certain amino acids may be substituted for other amino acids in a protein structure without appreciable loss of interactive binding capacity with structures such as, for example, antigen-binding regions of antibodies or binding sites on substrate molecules. Since it is the interactive capacity and nature of a protein that defines that protein's biological functional activity, certain amino acid sequence substitutions can be made in a protein sequence, and, of course, its underlying DNA coding sequence, and nevertheless obtain a protein with like properties. It is thus contemplated by the inventors that various changes may be made in the peptide sequences of the disclosed compositions, or corresponding DNA sequences which encode said peptides without appreciable loss of their biological utility or activity.

In making such changes, the hydropathic index of amino acids may be considered. The importance of the hydropathic amino acid index in conferring interactive biologic function on a protein is generally understood in the art (Kyte and

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Doolittle, 1982, incorporate herein by reference). It is accepted that the relative hydrophathic character of the amino acid contributes to the secondary structure of the resultant protein, which in turn defines the interaction of the protein with other molecules, for example, enzymes, substrates, receptors, DNA, antibodies, antigens, and the like.

Each amino acid has been assigned a hydropathic index on the basis of their hydrophobicity and charge characteristics (Kyte and Doolittle, 1982), these are: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5).

It is known in the art that certain amino acids may be substituted by other amino acids having a similar hydropathic index or score and still result in a protein with similar biological activity, *i.e.*, still obtain a biological functionally equivalent protein. In making such changes, the substitution of amino acids whose hydropathic indices are within  $\pm 2$  is preferred, those which are within  $\pm 1$  are particularly preferred, and those within  $\pm 0.5$  are even more particularly preferred.

It is also understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity. U.S. Patent 4,554,101, incorporated herein by reference, states that the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with a biological property of the protein.

As detailed in U.S. Patent 4,554,101, the following hydrophilicity values have been assigned to amino acid residues: arginine (+3.0); lysine (+3.0); aspartate (+3.0  $\pm$  1); glutamate (+3.0  $\pm$  1); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); threonine (-0.4); proline (-0.5  $\pm$  1); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5); tryptophan (-3.4).

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It is understood that an amino acid can be substituted for another having a similar hydrophilicity value and still obtain a biologically equivalent, and in particular, an immunologically equivalent protein. In such changes, the substitution of amino acids whose hydrophilicity values are within  $\pm 2$  is preferred, those which are within  $\pm 1$  are particularly preferred, and those within  $\pm 0.5$  are even more particularly preferred.

As outlined above, amino acid substitutions are generally therefore based on the relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. Exemplary substitutions which take various of the foregoing characteristics into consideration are well known to those of skill in the art and include: arginine and lysine; glutamate and aspartate; serine and threonine; glutamine and asparagine; and valine, leucine and isoleucine.

### 2.11 Site-Specific Mutagenesis

Site-specific mutagenesis is a technique useful in the preparation of individual peptides, or biologically functional equivalent proteins or peptides, through specific mutagenesis of the underlying DNA. The technique further provides a ready ability to prepare and test sequence variants, for example, incorporating one or more of the foregoing considerations, by introducing one or more nucleotide sequence changes into the DNA. Site-specific mutagenesis allows the production of mutants through the use of specific oligonucleotide sequences which encode the DNA sequence of the desired mutation, as well as a sufficient number of adjacent nucleotides, to provide a primer sequence of sufficient size and sequence complexity to form a stable duplex on both sides of the deletion junction being traversed. Typically, a primer of about 17 to 25 nucleotides in length is preferred, with about 5 to 10 residues on both sides of the junction of the sequence being altered.

In general, the technique of site-specific mutagenesis is well known in the art, as exemplified by various publications. As will be appreciated, the technique typically employs a phage vector which exists in both a single stranded and double stranded form. Typical vectors useful in site-directed mutagenesis include vectors



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such as the M13 phage. These phage are readily commercially available and their use is generally well known to those skilled in the art. Double stranded plasmids are also routinely employed in site directed mutagenesis which eliminates the step of transferring the gene of interest from a plasmid to a phage.

5        In general, site-directed mutagenesis in accordance herewith is performed by first obtaining a single-stranded vector or melting apart of two strands of a double stranded vector which includes within its sequence a DNA sequence which encodes the desired peptide. An oligonucleotide primer bearing the desired mutated sequence is prepared, generally synthetically. This primer is then annealed with the single-  
10        stranded vector, and subjected to DNA polymerizing enzymes such as *E. coli* polymerase I Klenow fragment, in order to complete the synthesis of the mutation-bearing strand. Thus, a heteroduplex is formed wherein one strand encodes the original non-mutated sequence and the second strand bears the desired mutation. This heteroduplex vector is then used to transform appropriate cells, such as *E. coli* cells,  
15        and clones are selected which include recombinant vectors bearing the mutated sequence arrangement.

      The preparation of sequence variants of the selected peptide-encoding DNA segments using site-directed mutagenesis is provided as a means of producing potentially useful species and is not meant to be limiting as there are other ways in  
20        which sequence variants of peptides and the DNA sequences encoding them may be obtained. For example, recombinant vectors encoding the desired peptide sequence may be treated with mutagenic agents, such as hydroxylamine, to obtain sequence variants.  
**2.12 Monoclonal Antibody Generation**

      Means for preparing and characterizing antibodies are well known in the art  
25        (See, e.g., Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, 1988; incorporated herein by reference). The methods for generating monoclonal antibodies (mAbs) generally begin along the same lines as those for preparing polyclonal antibodies. Briefly, a polyclonal antibody is prepared by immunizing an animal with an immunogenic composition in accordance with the present invention and collecting  
30        antisera from that immunized animal. A wide range of animal species can be used for

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the production of antisera. Typically the animal used for production of anti-antisera is a rabbit, a mouse, a rat, a hamster, a guinea pig or a goat. Because of the relatively large blood volume of rabbits, a rabbit is a preferred choice for production of polyclonal antibodies.

5       As is well known in the art, a given composition may vary in its immunogenicity. It is often necessary therefore to boost the host immune system, as may be achieved by coupling a peptide or polypeptide immunogen to a carrier. Exemplary and preferred carriers are keyhole limpet hemocyanin (KLH) and bovine serum albumin (BSA). Other albumins such as ovalbumin, mouse serum albumin or  
10       rabbit serum albumin can also be used as carriers. Means for conjugating a polypeptide to a carrier protein are well known in the art and include glutaraldehyde, *m*-maleimidobencoyl-*N*-hydroxysuccinimide ester, carbodiimide and bis-biazotized benzidine.

15       As is also well known in the art, the immunogenicity of a particular immunogen composition can be enhanced by the use of non-specific stimulators of the immune response, known as adjuvants. Exemplary and preferred adjuvants include complete Freund's adjuvant (a non-specific stimulator of the immune response containing killed *Mycobacterium tuberculosis*), incomplete Freund's adjuvants and aluminum hydroxide adjuvant.

20       The amount of immunogen composition used in the production of polyclonal antibodies varies upon the nature of the immunogen as well as the animal used for immunization. A variety of routes can be used to administer the immunogen (subcutaneous, intramuscular, intradermal, intravenous and intraperitoneal). The production of polyclonal antibodies may be monitored by sampling blood of the  
25       immunized animal at various points following immunization. A second, booster, injection may also be given. The process of boosting and titering is repeated until a suitable titer is achieved. When a desired level of immunogenicity is obtained, the immunized animal can be bled and the serum isolated and stored, and/or the animal can be used to generate mAbs.

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mAbs may be readily prepared through use of well-known techniques, such as those exemplified in U.S. Patent 4,196,265, incorporated herein by reference. Typically, this technique involves immunizing a suitable animal with a selected immunogen composition, *e.g.*, a purified or partially purified ACC protein, polypeptide or peptide. The immunizing composition is administered in a manner effective to stimulate antibody producing cells. Rodents such as mice and rats are preferred animals, however, the use of rabbit, sheep frog cells is also possible. The use of rats may provide certain advantages (Goding, 1986, pp. 60-61), but mice are preferred, with the BALB/c mouse being most preferred as this is most routinely used and generally gives a higher percentage of stable fusions.

Following immunization, somatic cells with the potential for producing antibodies, specifically B lymphocytes (B cells), are selected for use in the mAb generating protocol. These cells may be obtained from biopsied spleens, tonsils or lymph nodes, or from a peripheral blood sample. Spleen cells and peripheral blood cells are preferred, the former because they are a rich source of antibody-producing cells that are in the dividing plasmablast stage, and the latter because peripheral blood is easily accessible. Often, a panel of animals will have been immunized and the spleen of animal with the highest antibody titer will be removed and the spleen lymphocytes obtained by homogenizing the spleen with a syringe. Typically, a spleen from an immunized mouse contains approximately  $5 \times 10^7$  to  $2 \times 10^8$  lymphocytes.

The antibody-producing B lymphocytes from the immunized animal are then fused with cells of an immortal myeloma cell, generally one of the same species as the animal that was immunized. Myeloma cell lines suited for use in hybridoma-producing fusion procedures preferably are non-antibody-producing, have high fusion efficiency, and enzyme deficiencies that render them incapable of growing in certain selective media which support the growth of only the desired fused cells (hybridomas).

Any one of a number of myeloma cells may be used, as are known to those of skill in the art (Goding, pp. 65-66, 1986; Campbell, pp. 75-83, 1984). For example, where the immunized animal is a mouse, one may use P3-X63/Ag8, X63-Ag8.653,

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NS1/1.Ag 4 1, Sp210-Ag14, FO, NSO/U, MPC-11, MPC11-X45-GTG 1.7 and S194/5XX0 Bul; for rats, one may use R210.RCY3, Y3-Ag 1.2.3, IR983F and 4B210; and U-266, GM1500-GRG2, LICR-LON-HMy2 and UC729-6 are all useful in connection with human cell fusions.

5           One preferred murine myeloma cell is the NS-1 myeloma cell line (also termed P3-NS-1-Ag4-1), which is readily available from the NIGMS Human Genetic Mutant Cell Repository by requesting cell line repository number GM3573. Another mouse myeloma cell line that may be used is the 8-azaguanine-resistant mouse murine myeloma SP2/0 non-producer cell line.

10           Methods for generating hybrids of antibody-producing spleen or lymph node cells and myeloma cells usually comprise mixing somatic cells with myeloma cells in a 2:1 ratio, though the ratio may vary from about 20:1 to about 1:1, respectively, in the presence of an agent or agents (chemical or electrical) that promote the fusion of cell membranes. Fusion methods using Sendai virus have been described (Kohler and  
15       Milstein, 1975; 1976), and those using polyethylene glycol (PEG), such as 37% (v/v) PEG, (Geftter *et al.*, 1977). The use of electrically induced fusion methods is also appropriate (Goding, 1986, pp. 71-74).

          Fusion procedures usually produce viable hybrids at low frequencies, about  $1 \times 10^{-6}$  to  $1 \times 10^{-8}$ . However, this does not pose a problem, as the viable, fused  
20       hybrids are differentiated from the parental, unfused cells (particularly the unfused myeloma cells that would normally continue to divide indefinitely) by culturing in a selective medium. The selective medium is generally one that contains an agent that blocks the *de novo* synthesis of nucleotides in the tissue culture media. Exemplary and preferred agents are aminopterin, methotrexate, and azaserine. Aminopterin and  
25       methotrexate block *de novo* synthesis of both purines and pyrimidines, whereas azaserine blocks only purine synthesis. Where aminopterin or methotrexate is used, the media is supplemented with hypoxanthine and thymidine as a source of nucleotides (HAT medium). Where azaserine is used, the media is supplemented with hypoxanthine.

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The preferred selection medium is HAT. Only cells capable of operating nucleotide salvage pathways are able to survive in HAT medium. The myeloma cells are defective in key enzymes of the salvage pathway, *e.g.*, hypoxanthine phosphoribosyl transferase (HPRT), and they cannot survive. The B-cells can operate this pathway, but they have a limited life span in culture and generally die within about two weeks. Therefore, the only cells that can survive in the selective media are those hybrids formed from myeloma and B-cells.

This culturing provides a population of hybridomas from which specific hybridomas are selected. Typically, selection of hybridomas is performed by culturing the cells by single-clone dilution in microtiter plates, followed by testing the individual clonal supernatants (after about two to three weeks) for the desired reactivity. The assay should be sensitive, simple and rapid, such as radioimmunoassays, enzyme immunoassays, cytotoxicity assays, plaque assays, dot immunobinding assays, and the like.

The selected hybridomas would then be serially diluted and cloned into individual antibody-producing cell lines, which clones can then be propagated indefinitely to provide mAbs. The cell lines may be exploited for mAb production in two basic ways. A sample of the hybridoma can be injected (often into the peritoneal cavity) into a histocompatible animal of the type that was used to provide the somatic and myeloma cells for the original fusion. The injected animal develops tumors secreting the specific monoclonal antibody produced by the fused cell hybrid. The body fluids of the animal, such as serum or ascites fluid, can then be tapped to provide mAbs in high concentration. The individual cell lines could also be cultured *in vitro*, where the mAbs are naturally secreted into the culture medium from which they can be readily obtained in high concentrations. mAbs produced by either means may be further purified, if desired, using filtration, centrifugation and various chromatographic methods such as HPLC or affinity chromatography.

### 3. BRIEF DESCRIPTION OF THE DRAWINGS

The drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

FIG. 1. Structure of the cytosolic ACCase gene from wheat. Arrows indicate fragments of the genomic clones analyzed in more detail. Sequenced fragments are marked in black. The localization of the ACCase functional domains was established by amino acid sequence comparison with other biotin-dependent carboxylases (Gornicki *et al.*, 1994). BC, biotin carboxylase; BCC, biotin carboxyl carrier; CT, carboxyltransferase.

FIG. 2. Alignment of cDNA sequences corresponding to the 3'-end of the mRNA encoding wheat cytosolic ACCase. Only the sequence of the 3'-end of the RACE clones is shown. The putative polyadenylation signals are underlined. Asterisks indicate identical nucleotides. Sixteen additional 3'-RACE clones were sequenced, these matched one or another of the four sequences shown.

FIG. 3. DNA sequence of the wheat genomic ACC clone. The entire sequence is given in SEQ ID NO:30.

FIG. 4. Deduced amino acid sequence of the wheat genomic ACC clone shown in FIG. 3. The sequence is presented in SEQ ID NO:31.

FIG. 5. Shown is the 5' flanking sequence of the ACCase 1 gene (about 3 kb upstream of the translation initiation codon, of clone 71L. The sequence is shown in SEQ ID NO:32.

FIG. 6. Shown is the 5' flanking sequence of the ACCase 2 gene designated 153. The sequence is shown in SEQ ID NO:33.

### 4. DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

#### 4.1 Definitions

The following words and phrases have the meanings set forth below:

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*Expression:* The combination of intracellular processes, including transcription and translation undergone by a coding DNA molecule such as a structural gene to produce a polypeptide.

5     *Promoter:* A recognition site on a DNA sequence or group of DNA sequences that provide an expression control element for a structural gene and to which RNA polymerase specifically binds and initiates RNA synthesis (transcription) of that gene.

*Regeneration:* The process of growing a plant from a plant cell (e.g., plant protoplast or explant).

*Structural gene:* A gene that is expressed to produce a polypeptide.

10     *Transformation:* A process of introducing an exogenous DNA sequence (e.g., a vector, a recombinant DNA molecule) into a cell or protoplast in which that exogenous DNA is incorporated into a chromosome or is capable of autonomous replication.

15     *Transformed cell:* A cell whose DNA has been altered by the introduction of an exogenous DNA molecule into that cell.

*Transgenic cell:* Any cell derived or regenerated from a transformed cell or derived from a transgenic cell. Exemplary transgenic cells include plant calli derived from a transformed plant cell and particular cells such as leaf, root, stem, e.g., somatic cells, or reproductive (germ) cells obtained from a transgenic plant.

20     *Transgenic plant:* A plant or progeny thereof derived from a transformed plant cell or protoplast, wherein the plant DNA contains an introduced exogenous DNA molecule not originally present in a native, non-transgenic plant of the same strain. The terms "transgenic plant" and "transformed plant" have sometimes been used in the art as synonymous terms to define a plant whose DNA contains an exogenous DNA  
25     molecule. However, it is thought more scientifically correct to refer to a regenerated plant or callus obtained from a transformed plant cell or protoplast as being a transgenic plant, and that usage will be followed herein.

*Vector:* A DNA molecule capable of replication in a host cell and/or to which another DNA segment can be operatively linked so as to bring about replication of the  
30     attached segment. A plasmid is an exemplary vector.

## 4.2 Polynucleotides

Amino acid sequences of biotin carboxylase (BC) from *Anabaena* and *Synechococcus* show great similarity with amino acid residue sequences from other ACC enzymes as well as with the amino acid residue sequences of other biotin-containing enzymes. Based on that homology, specific nucleotide sequences were chosen for the construction of primers for polymerase chain reaction amplification of a corresponding region of the gene for ACC from wheat. Those primers have the nucleotide sequences shown below:

10      Primer 1    5'-TCGAATTCGTNATNATHAARGC-3' (SEQ ID NO:13);

Primer 2    5'-GCTCTAGAGKRTGYTCNACYTG-3' (SEQ ID NO:14);

where N is A, C, G or T; H is A, C or T; R is A or G; Y is T or C and K is G or T. Primers 1 and 2 comprise a 14-nucleotide specific sequence based on a conserved amino acid sequence and an 8-nucleotide extension at the 5'-end of the primer to provide anchors for rounds of amplification after the first round and to provide convenient restriction sites for analysis and cloning.

In eukaryotic ACCs, a BCCP domain is located about 300 amino acids away from the end of the BC domain, on the C-terminal side. Therefore, it is possible to amplify the cDNA covering the interval between the BC and BCCP domains using primers from the C-terminal end of the BC domain and the conserved MKM region of the BCCP. The BC primer was based on the wheat cDNA sequence obtained as described above. Those primers, each with 6- or 8-base 5'-extensions, are shown below:

25      Primer 3    5'-GCTCTAGAATACTATTTCCTG-3' (SEQ ID NO:15)

Primer 4    5'-TCGAATTCWNCATYTTTCATNRC-3' (SEQ ID NO:16)

where N, R and Y are as defined above. W is A or T. The BC primer (primer 3) was based on the wheat cDNA sequence obtained as described above. The MKM primer (primer 4) was first checked by determining whether it would amplify the *fabE* gene coding BCCP from *Anabaena* DNA. This PCR™ was primed at the other end by using a primer based on the N-terminal amino acid residue sequence as determined on



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protein purified from *Anabaena* extracts by affinity chromatography. Those primers are shown below:

Primer 5 5'-GCTCTAGAYTTYAAYGARATHMG-3' (SEQ ID NO:17)

Primer 4 5'-TCGAATTCWNCATYTTTCATNRC-3' (SEQ ID NO:18)

5        where H, N, R, T, Y and W are as defined above. M is A or C. This amplification (using the conditions described above) yielded the correct fragment of the *Anabaena fabE* gene, which was used to identify cosmids that contained the entire *fabE* gene and flanking DNA. An about 4-kb *Xba*I fragment containing the gene was cloned into the vector pBluescriptKS® for sequencing. Primers 3 and 4 were then  
10        used to amplify the intervening sequence in wheat cDNA. Again, the product of the first PCR™ was eluted and reamplified by another round of PCR™, then cloned into the Invitrogen vector pCRII®.

      The amino acid sequence of the polypeptide predicted from the cDNA sequence for this entire fragment of wheat cDNA (1473 nucleotides) was compared  
15        with the amino acid sequences of other ACC enzymes and related enzymes from various sources. Rat, chicken and yeast are more closely related to each other than to the BC subunits of bacteria, and the BC domains of other enzymes such as pyruvate carboxylase of yeast and propionyl CoA carboxylase of rat. The amino acid identities between wheat ACC and other biotin-dependent enzymes, within the BC domain are  
20        no higher than 60%, and shown below in Table 2.

TABLE 2

	% identity with wheat ACC	# identity with rat ACC
rat ACC	58	(100)
chicken ACC	57	
yeast ACC	56	
<i>Synechococcus</i> ACC	32	
<i>Anabaena</i> ACC	30	
<i>E. coli</i> ACC	33	
rat propionyl CoA carboxylase	32	31
yeast pyruvate carboxylase	31	

#### 4.3 Probes and Primers

In another aspect, DNA sequence information provided by the invention allows for the preparation of relatively short DNA (or RNA) sequences having the ability to specifically hybridize to gene sequences of the selected polynucleotides disclosed herein. In these aspects, nucleic acid probes of an appropriate length are prepared based on a consideration of a selected ACC gene sequence, *e.g.*, a sequence such as that shown in SEQ ID NO:9 or SEQ ID NO:19, or a selected gene sequence encoding a subunit of a cyanobacterial ACC, *e.g.*, a sequence as that shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, or SEQ ID NO:11. The ability of such nucleic acid probes to specifically hybridize to an ACC gene sequence lend them particular utility in a variety of embodiments. Most importantly, the probes can be used in a variety of assays for detecting the presence of complementary sequences in a given sample.

In certain embodiments, it is advantageous to use oligonucleotide primers. The sequence of such primers is designed using a polynucleotide of the present invention for use in detecting, amplifying or mutating a defined segment of an ACC gene from a cyanobacterium or a plant using PCR™ technology. Segments of ACC genes from other organisms may also be amplified by PCR™ using such primers.

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To provide certain of the advantages in accordance with the present invention, a preferred nucleic acid sequence employed for hybridization studies or assays includes sequences that are complementary to at least a 14 to 30 or so long nucleotide stretch of an ACC-encoding or ACC subunit-encoding sequence, such as that shown  
5 in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, or SEQ ID NO:19. A size of at least 14 nucleotides in length helps to ensure that the fragment will be of sufficient length to form a duplex molecule that is both stable and selective. Molecules having complementary sequences over stretches greater than 14 bases in length are generally preferred, though, in order to increase  
10 stability and selectivity of the hybrid, and thereby improve the quality and degree of specific hybrid molecules obtained. One will generally prefer to design nucleic acid molecules having gene-complementary stretches of 14 to 20 nucleotides, or even longer where desired. Such fragments may be readily prepared by, for example, directly synthesizing the fragment by chemical means, by application of nucleic acid  
15 reproduction technology, such as the PCR™ technology of U.S. Patents 4, 683,195, and 4,683,202, herein incorporated by reference, or by excising selected DNA fragments from recombinant plasmids containing appropriate inserts and suitable restriction sites.

Accordingly, a nucleotide sequence of the invention can be used for its ability  
20 to selectively form duplex molecules with complementary stretches of the gene. Depending on the application envisioned, one will desire to employ varying conditions of hybridization to achieve varying degree of selectivity of the probe toward the target sequence. For applications requiring a high degree of selectivity, one will typically desire to employ relatively stringent conditions to form the hybrids,  
25 for example, one will select relatively low salt and/or high temperature conditions, such as provided by about 0.02 M to about 0.15 M NaCl at temperatures of about 50°C to about 70°C. These conditions are particularly selective, and tolerate little, if any, mismatch between the probe and the template or target strand.

Of course, for some applications, for example, where one desires to prepare  
30 mutants employing a mutant primer strand hybridized to an underlying template or

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where one seeks to isolate an ACC coding sequences for related species, functional equivalents, or the like, less stringent hybridization conditions will typically be needed in order to allow formation of the heteroduplex. In these circumstances, one may desire to employ conditions such as about 0.15 M to about 0.9 M salt, at temperatures ranging from about 20°C to about 55°C. Cross-hybridizing species can thereby be readily identified as positively hybridizing signals with respect to control hybridizations. In any case, it is generally appreciated that conditions can be rendered more stringent by the addition of increasing amounts of formamide, which serves to destabilize the hybrid duplex in the same manner as increased temperature. Thus, hybridization conditions can be readily manipulated, and thus will generally be a method of choice depending on the desired results.

In certain embodiments, it is advantageous to employ a polynucleotide of the present invention in combination with an appropriate label for detecting hybrid formation. A wide variety of appropriate labels are known in the art, including radioactive, enzymatic or other ligands, such as avidin/biotin, which are capable of giving a detectable signal.

In general, it is envisioned that a hybridization probe described herein is useful both as a reagent in solution hybridization as well as in embodiments employing a solid phase. In embodiments involving a solid phase, the test DNA (or RNA) is adsorbed or otherwise affixed to a selected matrix or surface. This fixed nucleic acid is then subjected to specific hybridization with selected probes under desired conditions. The selected conditions depend as is well known in the art on the particular circumstances and criteria required (*e.g.*, on the G+C content, type of target nucleic acid, source of nucleic acid, size of hybridization probe). Following washing of the matrix to remove nonspecifically bound probe molecules, specific hybridization is detected, or even quantitated, by means of the label.

#### 4.4 Expression Vectors

The present invention contemplates an expression vector comprising a polynucleotide of the present invention. Thus, in one embodiment an expression

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vector is an isolated and purified DNA molecule comprising a promoter operatively linked to an coding region that encodes a polypeptide having the ability to catalyze the carboxylation of a biotin carboxyl carrier protein of a cyanobacterium, which coding region is operatively linked to a transcription-terminating region, whereby the  
5 promoter drives the transcription of the coding region.

As used herein, the term "operatively linked" means that a promoter is connected to an coding region in such a way that the transcription of that coding region is controlled and regulated by that promoter. Means for operatively linking a promoter to a coding region are well known in the art.

10 Where an expression vector of the present invention is to be used to transform a cyanobacterium, a promoter is selected that has the ability to drive and regulate expression in cyanobacteria. Promoters that function in bacteria are well known in the art. An exemplary and preferred promoter for the cyanobacterium *Anabaena* is the *glnA* gene promoter. An exemplary and preferred promoter for the cyanobacterium  
15 *Synechococcus* is the *psbAI* gene promoter. Alternatively, the cyanobacterial *acc* gene promoters themselves can be used.

Where an expression vector of the present invention is to be used to transform a plant, a promoter is selected that has the ability to drive expression in plants. Promoters that function in plants are also well known in the art. Useful in expressing  
20 the polypeptide in plants are promoters that are inducible, viral, synthetic, constitutive as described (Poszkowski *et al.*, 1989; Odell *et al.*, 1985), and temporally regulated, spatially regulated, and spatio-temporally regulated (Chau *et al.*, 1989).

A promoter is also selected for its ability to direct the transformed plant cell's or transgenic plant's transcriptional activity to the coding region. Structural genes can be driven by a variety of promoters in plant tissues. Promoters can be near-  
25 constitutive, such as the CaMV 35S promoter, or tissue-specific or developmentally specific promoters affecting dicots or monocots.

Where the promoter is a near-constitutive promoter such as CaMV 35S, increases in polypeptide expression are found in a variety of transformed plant tissues  
30 (*e.g.*, callus, leaf, seed and root). Alternatively, the effects of transformation can be

directed to specific plant tissues by using plant integrating vectors containing a tissue-specific promoter.

An exemplary tissue-specific promoter is the lectin promoter, which is specific for seed tissue. The Lectin protein in soybean seeds is encoded by a single gene (*Le1*) that is only expressed during seed maturation and accounts for about 2 to about 5% of total seed mRNA. The lectin gene and seed-specific promoter have been fully characterized and used to direct seed specific expression in transgenic tobacco plants (Vodkin *et al.*, 1983; Lindstrom *et al.*, 1990.)

An expression vector containing a coding region that encodes a polypeptide of interest is engineered to be under control of the lectin promoter and that vector is introduced into plants using, for example, a protoplast transformation method (Dhir *et al.*, 1991). The expression of the polypeptide is directed specifically to the seeds of the transgenic plant.

A transgenic plant of the present invention produced from a plant cell transformed with a tissue specific promoter can be crossed with a second transgenic plant developed from a plant cell transformed with a different tissue specific promoter to produce a hybrid transgenic plant that shows the effects of transformation in more than one specific tissue.

Exemplary tissue-specific promoters are corn sucrose synthetase 1 (Yang *et al.*, 1990), corn alcohol dehydrogenase 1 (Vogel *et al.*, 1989), corn light harvesting complex (Simpson, 1986), corn heat shock protein (Odell *et al.*, 1985), pea small subunit RuBP Carboxylase (Poulsen *et al.*, 1986; Cashmore *et al.*, 1983), Ti plasmid mannopine synthase (Langridge *et al.*, 1989), Ti plasmid nopaline synthase (Langridge *et al.*, 1989), petunia chalcone isomerase (Van Tunen *et al.*, 1988), bean glycine rich protein 1 (Keller *et al.*, 1989), CaMV 35s transcript (Odell *et al.*, 1985) and Potato patatin (Wenzler *et al.*, 1989). Preferred promoters are the cauliflower mosaic virus (CaMV 35S) promoter and the S-E9 small subunit RuBP carboxylase promoter.

The choice of which expression vector and ultimately to which promoter a polypeptide coding region is operatively linked depends directly on the functional properties desired, *e.g.*, the location and timing of protein expression, and the host cell

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to be transformed. These are well known limitations inherent in the art of constructing recombinant DNA molecules. However, a vector useful in practicing the present invention is capable of directing the expression of the polypeptide coding region to which it is operatively linked.

5        Typical vectors useful for expression of genes in higher plants are well known in the art and include vectors derived from the tumor-inducing (Ti) plasmid of *Agrobacterium tumefaciens* described (Rogers *et al.*, 1987). However, several other plant integrating vector systems are known to function in plants including pCaMVCN transfer control vector described (Fromm *et al.*, 1985). Plasmid pCaMVCN (available  
10        from Pharmacia, Piscataway, NJ) includes the cauliflower mosaic virus CaMV 35S promoter.

         In preferred embodiments, the vector used to express the polypeptide includes a selection marker that is effective in a plant cell, preferably a drug resistance selection marker. One preferred drug resistance marker is the gene whose expression  
15        results in kanamycin resistance; *i.e.*, the chimeric gene containing the nopaline synthase promoter, Tn5 neomycin phosphotransferase II and nopaline synthase 3' nontranslated region described (Rogers *et al.*, 1988).

         RNA polymerase transcribes a coding DNA sequence through a site where polyadenylation occurs. Typically, DNA sequences located a few hundred base pairs  
20        downstream of the polyadenylation site serve to terminate transcription. Those DNA sequences are referred to herein as transcription-termination regions. Those regions are required for efficient polyadenylation of transcribed messenger RNA (mRNA).

         Means for preparing expression vectors are well known in the art. Expression (transformation vectors) used to transform plants and methods of making those  
25        vectors are described in United States Patent Nos. 4,971,908, 4,940,835, 4,769,061 and 4,757,011, the disclosures of which are incorporated herein by reference. Those vectors can be modified to include a coding sequence in accordance with the present invention.

         A variety of methods has been developed to operatively link DNA to vectors  
30        via complementary cohesive termini or blunt ends. For instance, complementary

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homopolymer tracts can be added to the DNA segment to be inserted and to the vector DNA. The vector and DNA segment are then joined by hydrogen bonding between the complementary homopolymeric tails to form recombinant DNA molecules.

5 A coding region that encodes a polypeptide having the ability to catalyze the carboxylation of a biotin carboxyl carrier protein of a cyanobacterium is preferably a biotin carboxylase enzyme of a cyanobacterium, which enzyme is a subunit of acetyl-CoA carboxylase and participates in the carboxylation of acetyl-CoA. In a preferred embodiment, such a polypeptide has the amino acid residue sequence of SEQ ID NO:6 or SEQ ID NO:8, or a functional equivalent of those sequences. In accordance  
10 with such an embodiment, a coding region comprises the entire DNA sequence of SEQ ID NO:5 or the DNA sequence of SEQ ID NO:5 comprising the *Anabaena accC* gene. Alternatively, a coding region comprises the entire DNA sequence of SEQ ID NO:7 or the DNA sequence of SEQ ID NO:7 comprising the *Synechococcus accC* gene.

15 In another embodiment, an expression vector comprises a DNA segment that encodes a biotin carboxyl carrier protein of a cyanobacterium. That biotin carboxyl carrier protein preferably includes the amino acid residue sequence of SEQ ID NO:2 or SEQ ID NO:4, or functional equivalents thereof. In accordance with such an embodiment, a coding region comprises the entire DNA sequence of SEQ ID NO:1 or  
20 the DNA sequence of SEQ ID NO:1 comprising the *Anabaena accB* gene. Alternatively, a coding region comprises the entire DNA sequence of SEQ ID NO:3 or the DNA sequence of SEQ ID NO:3 comprising the *Synechococcus accB* gene.

In another embodiment, an expression vector comprises a DNA segment that encodes a carboxyltransferase protein of a cyanobacterium. That carboxyltransferase  
25 protein preferably includes a CT $\alpha$  or CT $\beta$  subunit, and preferably includes the amino acid residue sequence of SEQ ID NO:12, or a functional equivalent thereof. In accordance with such an embodiment, a coding region comprises the entire DNA sequence of SEQ ID NO:11 or the DNA sequence of SEQ ID NO:11 comprising the *Synechococcus accA* gene.



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In still yet another embodiment, an expression vector comprises a coding region that encodes a plant polypeptide having the ability to catalyze the carboxylation of acetyl-CoA. Such a plant polypeptide is preferably a monocotyledonous or a dicotyledonous plant acetyl-CoA carboxylase enzyme. A preferred monocotyledonous plant polypeptide encoded by such a coding region is preferably wheat ACC, which ACC includes the amino acid residue sequence of SEQ ID NO:10 or SEQ ID NO:31 or functional equivalents thereof. A preferred coding region includes the DNA sequence of SEQ ID NO:9 or SEQ ID NO:30. Alternatively, a preferred dicotyledonous plant ACC, such as canola ACC, is also preferred. Such an ACC enzyme is encoded by the DNA segment of SEQ ID NO:19 and has the amino acid sequence of SEQ ID NO:20.

#### 4.5 Polypeptides

The present invention provides novel polypeptides that define a whole or a portion of an ACC of a cyanobacterium or a plant. In one embodiment, thus, the present invention provides an isolated polypeptide having the ability to catalyze the carboxylation of a biotin carboxyl carrier protein of a cyanobacterium such as *Anabaena* or *Synechococcus*. Preferably, a biotin carboxyl carrier protein from *Anabaena* includes the amino acid sequence of SEQ ID NO:2, with such amino acid sequence listing encoded by the DNA segment of SEQ ID NO:1. Preferably, a biotin carboxyl carrier protein from *Synechococcus* includes the amino acid sequence of SEQ ID NO:4, with such amino acid sequence listing encoded by the DNA segment of SEQ ID NO:2.

In another embodiment, the present invention provides an isolated polypeptide comprising a biotin carboxylase protein of a cyanobacterium such as *Anabaena* or *Synechococcus*. Preferably, a biotin carboxylase protein from *Anabaena* includes the amino acid sequence of SEQ ID NO:6, with such amino acid sequence listing encoded by the DNA segment of SEQ ID NO:5. Preferably, a biotin carboxylase protein from *Synechococcus* includes the amino acid sequence of SEQ ID NO:8, with such amino acid sequence listing encoded by the DNA segment of SEQ ID NO:7.

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In another embodiment, the present invention provides an isolated polypeptide comprising a carboxyltransferase protein of a cyanobacterium such as *Synechococcus*.

Preferably, a carboxyltransferase protein comprises a CT $\alpha$  or CT $\beta$  subunit and includes the amino acid sequence of SEQ ID NO:12, with such amino acid sequence listing encoded by the DNA segment of SEQ ID NO:11.

In another embodiment, the present invention contemplates an isolated and purified plant polypeptide having a molecular weight of about 220 kDa, dimers of which have the ability to catalyze the carboxylation of acetyl-CoA. Such a polypeptide preferably includes the amino acid residue sequence of SEQ ID NO:10 or SEQ ID NO:31, with such amino acid sequence listing encoded by the DNA segment of SEQ ID NO:9 or SEQ ID NO:30. Alternatively the present invention provides an isolated and purified plant polypeptide from canola which has the ability to catalyze the carboxylation of acetyl-CoA. Such a polypeptide preferably includes the amino acid residue sequence of SEQ ID NO:20, with such amino acid sequence listing encoded by the DNA segment of SEQ ID NO:19.

#### 4.6 Transformed or Transgenic Cells or Plants

A cyanobacterium, a yeast cell, or a plant cell or a plant transformed with an expression vector of the present invention is also contemplated. A transgenic cyanobacterium, yeast cell, plant cell or plant derived from such a transformed or transgenic cell is also contemplated. Means for transforming cyanobacteria and yeast cells are well known in the art. Typically, means of transformation are similar to those well known means used to transform other bacteria or yeast such as *E. coli* or *Saccharomyces cerevisiae*. *Synechococcus* can be transformed simply by incubation of log-phase cells with DNA. (Golden *et al.*, 1987)

Methods for DNA transformation of plant cells include *Agrobacterium*-mediated plant transformation, protoplast transformation, gene transfer into pollen, injection into reproductive organs, injection into immature embryos and particle bombardment. Each of these methods has distinct advantages and disadvantages. Thus, one particular method of introducing genes into a particular plant strain may not

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necessarily be the most effective for another plant strain, but it is well known which methods are useful for a particular plant strain.

There are many methods for introducing transforming DNA segments into cells, but not all are suitable for delivering DNA to plant cells. Suitable methods are believed to include virtually any method by which DNA can be introduced into a cell, such as by *Agrobacterium* infection, direct delivery of DNA such as, for example, by PEG-mediated transformation of protoplasts (Omirulleh *et al.*, 1993), by desiccation/inhibition-mediated DNA uptake, by electroporation, by agitation with silicon carbide fibers, by acceleration of DNA coated particles, *etc.* In certain embodiments, acceleration methods are preferred and include, for example, microprojectile bombardment and the like.

Technology for introduction of DNA into cells is well-known to those of skill in the art. Four general methods for delivering a gene into cells have been described: (1) chemical methods (Graham and van der Eb, 1973; Zatloukal *et al.*, 1992); (2) physical methods such as microinjection (Capecchi, 1980), electroporation (Wong and Neumann, 1982; Fromm *et al.*, 1985) and the gene gun (Johnston and Tang, 1994; Fynan *et al.*, 1993); (3) viral vectors (Clapp, 1993; Lu *et al.*, 1993; Eglitis and Anderson, 1988a; 1988b); and (4) receptor-mediated mechanisms (Curiel *et al.*, 1991; 1992; Wagner *et al.*, 1992).

#### 4.6.1 Electroporation

The application of brief, high-voltage electric pulses to a variety of animal and plant cells leads to the formation of nanometer-sized pores in the plasma membrane. DNA is taken directly into the cell cytoplasm either through these pores or as a consequence of the redistribution of membrane components that accompanies closure of the pores. Electroporation can be extremely efficient and can be used both for transient expression of clones genes and for establishment of cell lines that carry integrated copies of the gene of interest. Electroporation, in contrast to calcium phosphate-mediated transfection and protoplast fusion, frequently gives rise to cell lines that carry one, or at most a few, integrated copies of the foreign DNA.

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The introduction of DNA by means of electroporation, is well-known to those of skill in the art. In this method, certain cell wall-degrading enzymes, such as pectin-degrading enzymes, are employed to render the target recipient cells more susceptible to transformation by electroporation than untreated cells. Alternatively, recipient cells are made more susceptible to transformation, by mechanical wounding. To effect transformation by electroporation one may employ either friable tissues such as a suspension culture of cells, or embryogenic callus, or alternatively, one may transform immature embryos or other organized tissues directly. One would partially degrade the cell walls of the chosen cells by exposing them to pectin-degrading enzymes (pectolyases) or mechanically wounding in a controlled manner. Such cells would then be recipient to DNA transfer by electroporation, which may be carried out at this stage, and transformed cells then identified by a suitable selection or screening protocol dependent on the nature of the newly incorporated DNA.

#### 4.6.2 Microprojectile Bombardment

A further advantageous method for delivering transforming DNA segments to plant cells is microprojectile bombardment. In this method, particles may be coated with nucleic acids and delivered into cells by a propelling force. Exemplary particles include those comprised of tungsten, gold, platinum, and the like.

An advantage of microprojectile bombardment, in addition to it being an effective means of reproducibly stably transforming monocots, is that neither the isolation of protoplasts (Cristou *et al.*, 1988) nor the susceptibility to *Agrobacterium* infection is required. An illustrative embodiment of a method for delivering DNA into maize cells by acceleration is a Biolistics Particle Delivery System, which can be used to propel particles coated with DNA or cells through a screen, such as a stainless steel or Nytex screen, onto a filter surface covered with corn cells cultured in suspension. The screen disperses the particles so that they are not delivered to the recipient cells in large aggregates. It is believed that a screen intervening between the projectile apparatus and the cells to be bombarded reduces the size of projectiles

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aggregate and may contribute to a higher frequency of transformation by reducing damage inflicted on the recipient cells by projectiles that are too large.

For the bombardment, cells in suspension are preferably concentrated on filters or solid culture medium. Alternatively, immature embryos or other target cells may  
5 be arranged on solid culture medium. The cells to be bombarded are positioned at an appropriate distance below the macroprojectile stopping plate. If desired, one or more screens are also positioned between the acceleration device and the cells to be bombarded. Through the use of techniques set forth herein one may obtain up to 1000 or more foci of cells transiently expressing a marker gene. The number of cells in a  
10 focus which express the exogenous gene product 48 hours post-bombardment often range from 1 to 10 and average 1 to 3.

In bombardment transformation, one may optimize the prebombardment culturing conditions and the bombardment parameters to yield the maximum numbers of stable transformants. Both the physical and biological parameters for bombardment  
15 are important in this technology. Physical factors are those that involve manipulating the DNA/microprojectile precipitate or those that affect the flight and velocity of either the macro- or microprojectiles. Biological factors include all steps involved in manipulation of cells before and immediately after bombardment, the osmotic adjustment of target cells to help alleviate the trauma associated with bombardment,  
20 and also the nature of the transforming DNA, such as linearized DNA or intact supercoiled plasmids. It is believed that pre-bombardment manipulations are especially important for successful transformation of immature embryos.

Accordingly, it is contemplated that one may wish to adjust various of the bombardment parameters in small scale studies to fully optimize the conditions. One  
25 may particularly wish to adjust physical parameters such as gap distance, flight distance, tissue distance, and helium pressure. One may also minimize the trauma reduction factors (TRFs) by modifying conditions which influence the physiological state of the recipient cells and which may therefore influence transformation and integration efficiencies. For example, the osmotic state, tissue hydration and the  
30 subculture stage or cell cycle of the recipient cells may be adjusted for optimum

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transformation. The execution of other routine adjustments will be known to those of skill in the art in light of the present disclosure.

*Agrobacterium*-mediated transfer is a widely applicable system for introducing genes into plant cells because the DNA can be introduced into whole plant tissues, thereby bypassing the need for regeneration of an intact plant from a protoplast. The use of *Agrobacterium*-mediated plant integrating vectors to introduce DNA into plant cells is well known in the art. See, for example, the methods described (Fraley *et al.*, 1985; Rogers *et al.*, 1987). Further, the integration of the Ti-DNA is a relatively precise process resulting in few rearrangements. The region of DNA to be transferred is defined by the border sequences, and intervening DNA is usually inserted into the plant genome as described (Spielmann *et al.*, 1986; Jorgensen *et al.*, 1987).

Modern *Agrobacterium* transformation vectors are capable of replication in *E. coli* as well as *Agrobacterium*, allowing for convenient manipulations as described (Klee *et al.*, 1985). Moreover, recent technological advances in vectors for *Agrobacterium*-mediated gene transfer have improved the arrangement of genes and restriction sites in the vectors to facilitate construction of vectors capable of expressing various polypeptide coding genes. The vectors described (Rogers *et al.*, 1987), have convenient multi-linker regions flanked by a promoter and a polyadenylation site for direct expression of inserted polypeptide coding genes and are suitable for present purposes. In addition, *Agrobacterium* containing both armed and disarmed Ti genes can be used for the transformations. In those plant strains where *Agrobacterium*-mediated transformation is efficient, it is the method of choice because of the facile and defined nature of the gene transfer.

*Agrobacterium*-mediated transformation of leaf disks and other tissues such as cotyledons and hypocotyls appears to be limited to plants that *Agrobacterium* naturally infects. *Agrobacterium*-mediated transformation is most efficient in dicotyledonous plants. Few monocots appear to be natural hosts for *Agrobacterium*, although transgenic plants have been produced in asparagus using *Agrobacterium* vectors as described (Bytebier *et al.*, 1987). Therefore, commercially important cereal grains such as rice, corn, and wheat must usually be transformed using alternative

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methods. However, as mentioned above, the transformation of asparagus using *Agrobacterium* can also be achieved (see, for example, Bytebier *et al.*, 1987).

A transgenic plant formed using *Agrobacterium* transformation methods typically contains a single gene on one chromosome. Such transgenic plants can be referred to as being heterozygous for the added gene. However, inasmuch as use of the word "heterozygous" usually implies the presence of a complementary gene at the same locus of the second chromosome of a pair of chromosomes, and there is no such gene in a plant containing one added gene as here, it is believed that a more accurate name for such a plant is an independent segregant, because the added, exogenous gene segregates independently during mitosis and meiosis.

More preferred is a transgenic plant that is homozygous for the added structural gene; *i.e.*, a transgenic plant that contains two added genes, one gene at the same locus on each chromosome of a chromosome pair. A homozygous transgenic plant can be obtained by sexually mating (selfing) an independent segregant transgenic plant that contains a single added gene, germinating some of the seed produced and analyzing the resulting plants produced for enhanced carboxylase activity relative to a control (native, non-transgenic) or an independent segregant transgenic plant.

It is to be understood that two different transgenic plants can also be mated to produce offspring that contain two independently segregating added, exogenous genes. Selfing of appropriate progeny can produce plants that are homozygous for both added, exogenous genes that encode a polypeptide of interest. Back-crossing to a parental plant and out-crossing with a non-transgenic plant are also contemplated.

Transformation of plant protoplasts can be achieved using methods based on calcium phosphate precipitation, polyethylene glycol treatment, electroporation, and combinations of these treatments (see, for example, Potrykus *et al.*, 1985; Lorz *et al.*, 1985; Fromm *et al.*, 1986; Uchimiya *et al.*, 1986; Callis *et al.*, 1987; Marcotte *et al.*, 1988).

Application of these systems to different plant strains depends upon the ability to regenerate that particular plant strain from protoplasts. Illustrative methods for the

regeneration of cereals from protoplasts are described (Fujimura *et al.*, 1985; Toriyama *et al.*, 1986; Yamada *et al.*, 1986; Abdullah *et al.*, 1986).

To transform plant strains that cannot be successfully regenerated from protoplasts, other ways to introduce DNA into intact cells or tissues can be utilized. For example, regeneration of cereals from immature embryos or explants can be effected as described (Vasil, 1988). In addition, "particle gun" or high-velocity microprojectile technology can be utilized. (Vasil, 1992)

Using that latter technology, DNA is carried through the cell wall and into the cytoplasm on the surface of small metal particles as described (Klein *et al.*, 1987; Klein *et al.*, 1988; McCabe *et al.*, 1988). The metal particles penetrate through several layers of cells and thus allow the transformation of cells within tissue explants.

Thus, the amount of a gene coding for a polypeptide of interest (*i.e.*, a polypeptide having carboxylation activity) can be increased in monocotyledonous plants such as corn by transforming those plants using particle bombardment methods (Maddock *et al.*, 1991). By way of example, an expression vector containing a coding region for a dicotyledonous ACC and an appropriate selectable marker is transformed into a suspension of embryonic maize (corn) cells using a particle gun to deliver the DNA coated on microprojectiles. Transgenic plants are regenerated from transformed embryonic calli that express ACC. Particle bombardment has been used to successfully transform wheat (Vasil *et al.*, 1992).

DNA can also be introduced into plants by direct DNA transfer into pollen as described (Zhou *et al.*, 1983; Hess, 1987; Luo *et al.*, 1988). Expression of polypeptide coding genes can be obtained by injection of the DNA into reproductive organs of a plant as described (Pena *et al.*, 1987). DNA can also be injected directly into the cells of immature embryos and the rehydration of desiccated embryos as described (Neuhaus *et al.*, 1987; Benbrook *et al.*, 1986).

The development or regeneration of plants from either single plant protoplasts or various explants is well known in the art (Weissbach and Weissbach, 1988). This regeneration and growth process typically includes the steps of selection of transformed cells, culturing those individualized cells through the usual stages of



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embryonic development through the rooted plantlet stage. Transgenic embryos and seeds are similarly regenerated. The resulting transgenic rooted shoots are thereafter planted in an appropriate plant growth medium such as soil.

5 The development or regeneration of plants containing the foreign, exogenous gene that encodes a polypeptide of interest introduced by *Agrobacterium* from leaf explants can be achieved by methods well known in the art such as described (Horsch *et al.*, 1985). In this procedure, transformants are cultured in the presence of a selection agent and in a medium that induces the regeneration of shoots in the plant strain being transformed as described (Fraley *et al.*, 1983).

10 This procedure typically produces shoots within two to four months and those shoots are then transferred to an appropriate root-inducing medium containing the selective agent and an antibiotic to prevent bacterial growth. Shoots that rooted in the presence of the selective agent to form plantlets are then transplanted to soil or other media to allow the production of roots. These procedures vary depending upon the particular plant strain employed, such variations being well known in the art.

15 Preferably, the regenerated plants are self-pollinated to provide homozygous transgenic plants, as discussed before. Otherwise, pollen obtained from the regenerated plants is crossed to seed-grown plants of agronomically important, preferably inbred lines. Conversely, pollen from plants of those important lines is used to pollinate regenerated plants.

20 A transgenic plant of the present invention containing a desired polypeptide is cultivated using methods well known to one skilled in the art. Any of the transgenic plants of the present invention can be cultivated to isolate the desired ACC or fatty acids which are the products of the series of reactions of which that catalyzed by ACC is the first.

25 A transgenic plant of this invention thus has an increased amount of an coding region (*e.g.*, gene) that encodes a polypeptide of interest. A preferred transgenic plant is an independent segregant and can transmit that gene and its activity to its progeny. A more preferred transgenic plant is homozygous for that gene, and transmits that gene to all of its offspring on sexual mating.

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Seed from a transgenic plant is grown in the field or greenhouse, and resulting sexually mature transgenic plants are self-pollinated to generate true breeding plants. The progeny from these plants become true breeding lines that are evaluated for, by way of example, herbicide resistance, preferably in the field, under a range of environmental conditions.

5 The commercial value of a transgenic plant with increased herbicide resistance or with altered fatty acid production is enhanced if many different hybrid combinations are available for sale. The user typically grows more than one kind of hybrid based on such differences as time to maturity, standability or other agronomic  
10 traits. Additionally, hybrids adapted to one part of a country are not necessarily adapted to another part because of differences in such traits as maturity, disease and herbicide resistance. Because of this, herbicide resistance is preferably bred into a large number of parental lines so that many hybrid combinations can be produced.

#### 15 4.7 Process of Increasing Herbicide Resistance

Herbicides such as aryloxyphenoxypropionates and cyclohexane-1,3-dione derivatives inhibit the growth of monocotyledonous weeds by interfering with fatty acid biosynthesis of herbicide sensitive plants. ACC is the target enzyme for those herbicides. Dicotyledonous plants, other eukaryotic organisms and prokaryotic  
20 organisms are resistant to those compounds.

Thus, the resistance of sensitive monocotyledonous plants to herbicides can be increased by providing those plants with ACC that is not sensitive to herbicide inhibition. The present invention therefore provides a process of increasing the herbicide resistance of a monocotyledonous plant comprising transforming the plant  
25 with a DNA molecule comprising a promoter operatively linked to a coding region that encodes a herbicide resistant polypeptide having the ability to catalyze the carboxylation of acetyl-CoA, which coding region is operatively linked to a transcription-terminating region, whereby the promoter is capable of driving the transcription of the coding region in a monocotyledonous plant.

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Preferably, a herbicide resistant polypeptide, a dicotyledonous plant polypeptide such as an acetyl-CoA carboxylase enzyme from soybean, rape, sunflower, tobacco, *Arabidopsis*, petunia, canola, pea, bean, tomato, potato, lettuce, spinach, alfalfa, cotton or carrot, or functional equivalent thereof. A promoter and a transcription-terminating region are preferably the same as set forth above.

Transformed monocotyledonous plants can be identified using herbicide resistance. A process for identifying a transformed monocotyledonous plant cell involves transforming the monocotyledonous plant cell with a DNA molecule that encodes a dicotyledonous acetyl-CoA carboxylase enzyme, and determining the resistance of the plant cell to a herbicide and thereby the identification of the transformed monocotyledonous plant cell. Means for transforming a monocotyledonous plant cell are the same as set forth above.

The resistance of a transformed plant cell to a herbicide is preferably determined by exposing such a cell to an effective herbicidal dose of a preselected herbicide and maintaining that cell for a period of time and under culture conditions sufficient for the herbicide to inhibit ACC, alter fatty acid biosynthesis or retard growth. The effects of the herbicide can be studied by measuring plant cell ACC activity, fatty acid synthesis or growth.

An effective herbicidal dose of a given herbicide is that amount of the herbicide that retards growth or kills plant cells not containing herbicide-resistant ACC or that amount of a herbicide known to inhibit plant growth. Means for determining an effective herbicidal dose of a given herbicide are well known in the art. Preferably, a herbicide used in such a process is an aryloxyphenoxypropionate or cyclohexanedione herbicide.

25

#### 4.8 Process of Altering ACC Activity

ACC catalyzes the carboxylation of acetyl-CoA. Thus, the carboxylation of acetyl-CoA in a cyanobacterium or a plant can be altered by, for example, increasing an ACC gene copy number or changing the composition (*e.g.*, nucleotide sequence) of an ACC gene. Changes in ACC gene composition may alter gene expression at either

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the transcriptional or translational level. Alternatively, changes in gene composition can alter ACC function (*e.g.*, activity, binding) by changing primary, secondary or tertiary structure of the enzyme. By way of example, certain changes in ACC structure are associated with changes in the resistance of that altered ACC to herbicides. The copy number of such a gene can be increased by transforming a

5 DNA molecule that encodes ACC.

In one embodiment, therefore, the present invention contemplates a process of altering the carboxylation of acetyl-CoA in a cell comprising transforming the cell

10 with a DNA molecule comprising a promoter operatively linked to a coding region that encodes a polypeptide having the ability to catalyze the carboxylation of acetyl-CoA, which coding region is operatively linked to a transcription-terminating region, whereby the promoter is capable of driving the transcription of the coding region in the cyanobacterium.

15 In a preferred embodiment, a cell is a cyanobacterium or a plant cell, a polypeptide is a cyanobacterial ACC or a plant ACC. Exemplary and preferred expression vectors for use in such a process are the same as set forth above.

#### 4.9 Determining Herbicide Resistance Inheritability

20 In yet another aspect, the present invention provides a process for determining the inheritance of plant resistance to herbicides of the aryloxyphenoxypropionate or cyclohexanedione class. That process involves measuring resistance to herbicides of the aryloxyphenocypionate or cyclohexanedione class in a parental plant line and in progeny of the parental plant line and detecting the presence of a DNA segment

25 encoding ACC in such plants.

The inheritability of phenotypic traits such as herbicide resistance can be determined using RFLP analysis. Restriction fragment length polymorphisms (RFLPs) are due to sequence differences detectable by lengths of DNA fragments generated by digestion with restriction enzymes and typically revealed by agarose gel

30 electrophoresis. There are large numbers of restriction endonucleases available,

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characterized by their recognition sequences and source. From these studies, it is possible to correlate herbicide resistance with a particular DNA fragment and analyze the inheritance of such resistance in progeny plants.

In a preferred embodiment, the herbicide resistant variant of acetyl-CoA carboxylase is a dicotyledonous plant acetyl-CoA carboxylase enzyme or a portion thereof. In another preferred embodiment, the herbicide resistant variant of acetyl-CoA carboxylase is a mutated monocotyledonous plant acetyl-CoA carboxylase that confers herbicide resistance or a hybrid acetyl-CoA carboxylase comprising a portion of a dicotyledonous plant acetyl-CoA carboxylase, a portion of a monocotyledonous plant acetyl-CoA carboxylase or one or more domains of a cyanobacterial acetyl-CoA carboxylase.

Restriction fragment length polymorphism analyses are conducted, for example, by Native Plants Incorporated (NPI). This service is available to the public on a contractual basis. For this analysis, the genetic marker profile of the parental inbred lines is determined. If parental lines are essentially homozygous at all relevant loci (*i.e.*, they should have only one allele at each locus), the diploid genetic marker profile of the hybrid offspring of the inbred parents should be the sum of those parents, *e.g.*, if one parent had the allele A at a particular locus, and the other parent had B, the hybrid AB is by inference.

Probes capable of hybridizing to specific DNA segments under appropriate conditions are prepared using standard techniques well known to those skilled in the art. The probes are labelled with radioactive isotopes or fluorescent dyes for ease of detection. After restriction fragments are separated by size, they are identified by hybridization to the probe. Hybridization with a unique cloned sequence permits the identification of a specific chromosomal region (locus). Because all alleles at a locus are detectable, RFLP's are co-dominant alleles. They differ from some other types of markers, *e.g.*, from isozymes, in that they reflect the primary DNA sequence, they are not products of transcription or translation.

#### 4.10 Oil Content of Seeds

Manipulation of the oil content and quality of seeds may benefit from knowledge of this gene's structure and regulation. Understanding the basis of resistance to herbicides, on the other hand, will be useful for future attempts to construct transgenic grasses and to provide crop plants such as wheat with selective resistance.

Genes of the present invention may be introduced into plants, particularly monocotyledonous plants, particularly commercially important grains. A wide range of novel transgenic plants produced in this manner may be envisioned depending on the particular constructs introduced into the transgenic plants. The largest use of grain is for feed or food. Introduction of genes that alter the composition of the grain may greatly enhance the feed or food value.

The introduction of genes encoding ACC may alter the oil content of the grain, and thus may be of significant value. Increases in oil content may result in increases in metabolizable-energy-content and -density of the seeds for uses in feed and food. The introduction of genes such as ACC which encode rate-limiting enzymes in fatty acid biosynthesis, or replacement of these genes through gene disruption or deletion mutagenesis could have significant impact on the quality and quantity of oil in such transgenic plants.

Likewise, the introduction of the ACC genes of the present invention may also alter the balance of fatty acids present in the oil providing a more healthful or nutritive feedstuff. Alternatively, oil properties may also be altered to improve its performance in the production and use of cooking oil, shortenings, lubricants or other oil-derived products or improvement of its health attributes when used in the food-related applications. Such changes in oil properties may be achieved by altering the type, level, or lipid arrangement of the fatty acids present in the oil. This in turn may be accomplished by the addition of genes that encode enzymes that catalyze the synthesis of novel fatty acids and the lipids possessing them or by increasing levels of native fatty acids while possibly reducing levels of precursors.

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Alternatively, introduction of DNA segments which are complementary to the DNA segments disclosed herein into plant cells may bring about a decrease in ACC activity *in vivo* and lower the level of fatty acid biosynthesis in such transformed cells.

Therefore, transgenic plants containing such novel constructs may be important due to their decreased oil content in such cells. Introduction of specific mutations in either the DNA segments disclosed, or in their complements, may result in transformed plants having intermediate ACC activity.

The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

## 5. EXAMPLES

### 5.1 EXAMPLE 1 -- Cloning and Sequencing of the *Anabaena acc* Genes

#### 5.1.1 Biotin Carboxylase (*accC*)

The gene for the BC subunit was cloned with a fragment of the *E. coli fabG* gene as a heterologous hybridization probe. Southern analysis of *Anabaena* sp. strain PCC 7120 DNA digested with various restriction enzymes, carried out at low stringency (57°C, 1 M NaCl, GeneScreen Plus® membrane [DuPont]) in accordance with the manufacturer's protocol, with an *Sst*II-*Pst*I fragment consisting of ~90% of the coding region of the *fabG* gene from *E. coli* as a probe revealed, in each case, only one strongly hybridizing restriction fragment. The 3.1-kb *Hind*III fragment identified by this probe in the *Anabaena* sp. strain PCC 7120 DNA digest was purified by gel electrophoresis and then was digested with *Nhe*I, yielding a 1.6-kb *Nhe*I-*Hind*III fragment that hybridized with the same *fabG* probe. The 1.6-kb fragment was purified

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by gel electrophoresis and cloned into *XbaI-HindIII*-digested pUC18. The ends of the insert were sequenced.

5 A fragment of an open reading frame coding for a polypeptide with very high similarity to an internal sequence of *E. coli* BC was found at the *NheI* end of the insert. This result indicated that the 3.1-kb *HindIII* fragment contained the entire *Anabaena* sp. strain PCC 7120 BC gene. The 1.6-kb *Anabaena* sp strain PCC 7120 DNA fragment was then used as a probe to screen, at high stringency (65°C, 1 M NaCl), a cosmid library of *Anabaena* sp. strain PCC 7120 DNA in the cosmid vector pWB79 (Chang *et al.*, 1992), constructed by W.J. Buikema (University of Chicago) with a sized partial *HindIII* digest of chromosomal DNA. Five cosmids containing overlapping fragments of *Anabaena* sp. strain PCC 7120 DNA were found in the 1,920-member bank, all of which contained the same size *HindIII* and *NheI* fragments as those identified by the *E. coli* probe previously. From one of the cosmids, the 3.1-kb *HindIII* fragment was subcloned into pUC18 and sequenced.

15 Nucleotide sequences of both strands were determined on double-stranded templates by the dideoxy chain termination method with Sequenase (United States Biochemicals). Sets of nested deletions generated with an Erase-a-Base kit (Promega) as well as specific primers were used for sequencing. The 3065-nucleotide DNA segment comprising the *Anabaena accC* gene is given in SEQ ID NO:5. The 477-amino acid translation of the *accC* gene encoding the *Anabaena* BC protein is given in  
20 SEQ ID NO:6.

### 5.1.2 Biotin Carboxyl Carrier Protein (*accB*)

A different approach had to be used to clone the *Anabaena* sp. strain PCC 7120 BCCP gene. An earlier attempt to clone the gene with a fragment of *E. coli* DNA containing the *fabE* gene as a heterologous hybridization probe failed. Furthermore, analysis of the sequence (~1.3-kb) located upstream of the *Anabaena* sp. strain PCC 7120 BC gene revealed no open reading frame corresponding to BCCP, in contrast to the *E. coli* gene organization in which the BCCP gene is located  
25



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immediately upstream of the BC gene. The BCCP gene was cloned by PCR<sup>TM</sup> amplification.

The N-terminal amino acid sequence of BCCP was used to design an upstream PCR<sup>TM</sup> primer. The downstream primer was targeted to the conserved sequence  
5 encoding the biotinylation site. The primers had the following structure:

Amino acid sequence: LDFNEIR (SEQ ID NO:22)

Primer I 5'-GCTCTAGAYTTYAAAYGARATHMG-3' (SEQ ID NO:23)

Amino acid sequence: NMKMX (SEQ ID NO:24) (N= V or A)

Primer II 3'-CRNTACTTYTACNWCTTAAGCT-5' (SEQ ID NO:25)

10

where Y= T or C; R= A or G; M= C or A; H= A, C, or T; W= A or T; N= T, C, A, or G.

PCR<sup>TM</sup> was carried out as described in the GeneAmp® kit manual (Perkin-Elmer Cetus). All components of the PCR<sup>TM</sup> except the *Taq* DNA polymerase  
15 were incubated for 3 to 5 min at 95°C. The PCR<sup>TM</sup> was then initiated by the addition of polymerase. Amplification was for 45 cycles, each 1 min at 95°C, 1 min at 42 to 45°C, and 2 min at 72°C, with 0.5 to 1.0 µg of template DNA per ml and 50 µg of each primer per ml. The PCR<sup>TM</sup> amplification yielded a product ~450 bp in size (*i.e.*,  
the correct size for the anticipated fragment of the *Anabaena* sp. strain PCC 7120  
20 BCCP gene deduced from the *E. coli* sequence and allowing for a 60- to 90-nucleotide addition due to the polypeptide length difference). The PCR<sup>TM</sup> product was cloned into the Invitrogen vector pCR1000 with the A/T tail method and was sequenced to confirm its identity.

The fragment of the *Anabaena* sp. strain PCC 7120 BCCP gene was then used  
25 as a probe to identify cosmids that contain the entire gene and flanking DNA. Three such cosmids were detected in a 1,920-member library (same as described above). A 4.2-kb *Xba*I fragment containing the BCCP gene was subcloned into pBluescriptII®, and its *Hind*III-*Nhe*I fragment was sequenced with specific primers as described above. The 1458-nucleotide DNA segment comprising the *Anabaena accB* gene is

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given in SEQ ID NO:1. The 182-amino acid translation of the *accB* gene encoding the *Anabaena* BCCP is given in SEQ ID NO:2.

The amino acid sequence deduced from the DNA sequence of the BCCP gene exactly matches the N-terminal sequence obtained for purified protein. Likely translation initiation codons were identified by comparison with *E. coli*. For the BC gene, the AUG start codon is not preceded by an obvious ribosome-binding site. There is a stop codon in the same open reading frame one codon upstream from the AUG codon, excluding the possibility of additional amino acids at the N terminus. The GUG start codon for BCCP immediately precedes codons for the amino acids identified by protein sequencing of the N terminus of purified BCCP. A putative 5-nucleotide ribosome-binding site, GAGGU, is located 11 nucleotides upstream of the GUG codon. The open reading frame extends further upstream of the GUG codon (for about 60 codons), but there are no AUG or GUG codons that could serve as start sites from translation. This excludes the possibility that the purified BCCP polypeptide lacks more than one amino acid (Met) because of rapid proteolytic degradation.

Structural similarities deduced from the available amino acid sequences suggest strong evolutionary conservation among BCs (Al-Feel *et al.*, 1992; Knowles, 1989; Lopez-Casillas *et al.*, 1988; Samols *et al.*, 1988; Takai *et al.*, 1988). Comparison of the amino acid sequence of the BC domain defined as the part of the sequence between amino acids Lys-5 and Phe-432 of *Anabaena* sp. strain PCC 7120 BC, the two outermost amino acids present in all or all but one of the compared sequences, revealed that all highly conserved amino acid residues identified before are present in *Anabaena* sp. strain PCC 7120 BC, including the ATP binding site motif and the conserved sequence including Cys-230 as a part of the bicarbonate binding site. The identity between the amino acid sequence of the *Anabaena* sp. strain PCC 7120 BC domain (based on the best multiple alignment) and that of rat (Lopez-Casillas *et al.*, 1988), chicken (Takai *et al.*, 1988), yeast (Al-Feel *et al.*, 1992), and wheat ACCs was no more than 32 to 37%. Mitochondrial enzymes, rat propionyl-CoA carboxylase (Browner *et al.*, 1989) and yeast pyruvate carboxylase

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(Lim *et al.*, 1988), are only 45 to 47% identical. Similarities with carbamoyl-phosphate synthetases observed for other BCs (Knowles, 1989; Li and Cronan, 1992; Lopez-Casillas *et al.*, 1988; Samols *et al.*, 1988; Takai *et al.*, 1988) are also evident for *Anabaena* sp. strain PCC 7120 BC.

5        *Anabaena* sp. strain PCC 7120 BCCP is unique with its biotinylation site, the result of a single A-to-C base change resulting in a Met-to-Leu substitution. This base change explains the highly variable yield of the PCR™ amplification with primer II. The structure of this part of the BCCP gene was confirmed by sequencing the corresponding PCR™-cloned fragment of *Anabaena* sp. strain PCC 7120 DNA. The  
10 result is not entirely surprising, because *in vitro* analysis of mutants of the 1.3S subunit of transcarboxylase from *Propionibacterium shermanii*, in which the same Met-to-Leu change was introduced, showed that this methionine residue is not essential for efficient biotinylation of the apoprotein (Shenoy *et al.*, 1992). Urea carboxylase contains Ala at this position. The conserved motif may be required for  
15 some other functions. Furthermore, it was suggested that the distance between the biotinylated lysine residue and the C terminus and the structure of the last two amino acids (hydrophobic one followed by acidic one) are important determinants for the modification of at least some BCCP apoproteins (Shenoy *et al.*, 1992). Two amino acids with the same properties are also found at an analogous position (with respect to  
20 the distance from the biotinylation site) of large eukaryotic biotin-dependent carboxylases. *Anabaena* sp. strain PCC 7120 BCCP also contains those amino acids, but they are separated from the biotinylation site by two additional amino acids. *Anabaena* sp. strain PCC 7120 BCCP is about 30 amino acids longer than the *E. coli* protein, including a 21-amino-acid insertion near the N terminus. The moderate  
25 conservation of the amino acid sequence is reflected by rather low conservation at the nucleotide level (Table 3), which explains why the *E. coli* BCCP specific probe failed to identify the *Anabaena* sp. strain PCC 7120 gene.

Comparison of the amino acid sequence encoded by the additional short open reading frame located upstream of the BCCP gene and transcribed in the same

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direction and sequences deposited in GenBank (release 75) revealed no similar proteins.

### 5.1.3 Northern analysis of the BCCP message

5       The size of *Anabaena* sp. strain PCC 7120 BCCP mRNA was established by Northern (RNA) analysis with the PCR<sup>TM</sup>-amplified fragment of the gene as a probe. The major hybridizing mRNA is 1.45-kb in size. The two minor species are 1.85 and 2.05-kb in size. All of these are long enough to include the BCCP coding region. The amount of all three mRNAs seems to be higher (about twofold) in cells grown in the  
10       absence of combined nitrogen. The 24-h induction time correlates with the onset of nitrogen fixation in heterocysts, differentiated cells that fix nitrogen and have a unique glycolipid envelope containing C<sub>26</sub> and C<sub>28</sub> fatty acids (Murata and Nishida, 1987). If the increase of the level of the BCCP mRNA is heterocyst specific, it must be significant because heterocysts in *Anabaena* sp. strain PCC 7120 filaments are formed  
15       only at ~10-cell intervals. This result suggests that ACC may be developmentally regulated in *Anabaena* sp. strain PCC 7120. Results of some recent experiments indicate that, in bacteria, modulation of ACC activity may indeed play an important role in the overall regulation of the biosynthesis of the cell lipids. It has been demonstrated that the level of transcription of the ACC genes is correlated in *E. coli*  
20       with the rate of cellular growth and nutritional upshifts and downshifts (Li and Cronan, 1993). Mutations in the *E. coli fabGE* operon which decrease the rate of phospholipid biosynthesis suppress a null mutation in the *htrB* gene by restoring the balance between phospholipid biosynthesis and cell growth (Karow *et al.*, 1992). Northern analysis with the 1.6-kb *NheI-HindIII* fragment as a BC-specific probe  
25       repeatedly gave a smeared band pattern which could not be interpreted.

      Unlike the BCCP and BC genes of *E. coli* where they are cotranscribed, the BCCP and BC genes of the present invention are separated by at least several kilobases (no overlapping cosmids were seen when the cosmid library was screened with probes specific for BCCP and BC).

## 5.2 EXAMPLE 2 -- Purification and Characterization of *Anabaena* BCCP

Western immunoblot analysis of *Anabaena* sp. strain PCC 7120 proteins with <sup>35</sup>S-streptavidin revealed one biotinylated polypeptide ~25 kDa in size. Although the presence of other, much less abundant biotinylated proteins cannot be strictly ruled out, this result strongly suggests that ACC is the only biotin-dependent enzyme in *Anabaena* sp. strain PCC 7120, with the BCCP subunit of 19 kDa, the calculated size; 25 kDa as measured by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

The polypeptide shows a slightly lower mobility than *E. coli* BCCP (~22.5 kDa), suggesting that *Anabaena* sp. strain PCC 7120 BCCP is longer by 20 to 30 amino acids. However, the unusual electrophoretic properties of the *E. coli* protein (Li and Cronan, 1992) make an accurate prediction of the polypeptide length difficult.

Separation of *Anabaena* sp. strain PCC 7120 proteins for Western analysis or sequencing) was by SDS-PAGE with 12.5% separating gels (Sambrook *et al.*, 1989) followed by transfer onto polyvinylidene difluoride membrane (Immobilon-P®; Millipore) in 10 mM sodium 3-(cyclohexylamino)-1-propane-sulfonate buffer (pH 11)-10% methanol. Western blots were blocked with 3% bovine serum albumin solution in 10 mM Tris-HCl (pH 7.5) and 0.9% NaCl and then were incubated for 3 to 16 h with <sup>35</sup>S-streptavidin (Amersham). The blots were washed at room temperature with 0.5% Nonidet P-40™ in 10 mM Tris-HCl (pH 7.5) and 0.9% NaCl.

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**TABLE 3**  
**COMPARISON OF BC AND BCCP SUBUNITS FROM**  
*Anabaena* AND *E. coli*

ACC subunit <sup>a</sup>	No. of amino acids (mol wt) <sup>b</sup>		% Identity (similarity)
	<i>Anabaena</i> PCC 7120	<i>E. coli</i> <sup>c</sup>	
BC			
Protein	447 (49,076)	449	57 (74)
DNA <sup>d</sup>			58
BCCP			
Protein	182 (19,126)	156	39 (65)
DNA <sup>d</sup>			41

5     <sup>a</sup> The genes for the two subunits of ACC are unlinked in *Anabaena* sp. strain PCC 7120; in *E. coli* they are in one operon.

<sup>b</sup> Molecular weight was calculated from amino acid composition.

<sup>c</sup> From Li and Cronan, 1992.

<sup>d</sup> On the basis of amino acid alignment.

10

      BCCP from *Anabaena* sp. strain PCC 7120 was purified starting with cells from a 3-liter culture grown on BG11 medium (Rippka *et al.*, 1979). Cells were broken by sonication at 0°C in 30 ml of 0.5 M NaCl-0.1 M Tris-HCl (pH 7.5)-14 mM β-mercaptoethanol-0.2 mM phenylmethylsulfonyl fluoride. Insoluble material was removed by centrifugation at 31,000 × g for 30 min, and the soluble protein fraction containing BCCP was precipitated by adding solid ammonium sulfate (50% saturation). The pellet was resuspended in 15 ml of 0.2 M NaCl-50 mM Tris-HCl (pH 7.5)-10% glycerol-0.5% SDS and then mixed at room temperature for about 18 h with 0.5 ml of streptavidin-agarose suspension (GIBCO BRL). The mixture was loaded onto a column, was washed with about 30 ml of 0.25 M NaCl-50 mM Tris-HCl (pH 7.5)-0.5 mM EDTA-0.2% SDS, and then was washed with 5 ml of water. Biotinylated peptides were eluted with 3 ml of 70% formic acid, dried under vacuum,

20

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and separated by SDS-PAGE. The N-terminal sequence of the biotin-containing ~25-kDa polypeptide was determined by Edman degradation after transfer to Immobilon-P® as described above. The sequence was PLDFNEIRQL (SEQ ID NO:21).

5

### 5.3 EXAMPLE 3 -- Characterization of the *Synechococcus acc* Genes and Purification of the *Synechococcus* BCCP

#### 5.3.1 Biotin Carboxylase (*accC*)

All carboxylases have a conserved amino acid motif that constitutes the ATP-binding site. A 1.2-kb *Sst*II-*Pst*I fragment (containing the ATP-binding motif) within the *E. coli accC* gene was used as a probe to examine the *Synechococcus* PCC 7942 genomic DNA by Southern hybridization at 58°C. A strongly hybridizing 0.8-kb *Bam*HI-*Pst*I fragment was detected and subsequently cloned by a two-stage size fractionation method.

15 *Synechococcus* PCC 7942 genomic DNA was first digested with *Bam*HI and electrophoresed on an agarose gel. The gel region containing DNA of sizes between 1.6-kb and 3-kb was cut out and purified (using GeneClean II Kit from Bio101). The purified DNA was then digested with *Pst*I and electrophoresed on an agarose gel. The gel region containing DNA of sizes between 0.5-kb and 2-kb was cut out and purified.

20 DNA samples (from each step of purification) were electrophoresed, transferred onto a Genescreen Plus membrane, hybridized with the *E. coli accC* probe to confirm that the homologous DNA fragment was not lost during each purification step. A library of fragments between 0.5-kb and 2-kb was created by cloning the purified fraction of *Synechococcus* PCC 7942 DNA into vector pBluescript® KS. Ampicillin-resistant

25 and white (*i.e.*, with insert) colonies were selected by plating on LB plates containing ampicillin, X-Gal and IPTG.

A total of 287 ampicillin-resistant, white clones were screened; the plasmid DNA mixture (from pools of 5 white clones per pool) were prepared, doubly-digested with *Pst*I and *Bam*HI, electrophoresed, transferred onto a Genescreen Plus membrane,

30 then hybridized with the *E. coli accC* probe at 58°C. Positive signals appeared on 8

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pools. Twelve positive individual clones were identified at the second round of screening. Two (of the 12) positive clones, each with a single fragment inserted, had the inserts sequenced. Both clones had identical inserts. Sequence comparison indicated only about 60% identity at the nucleotide level between the *E. coli accC* gene and the cloned *Synechococcus PstI-BamHI* fragment. This cloned fragment was then used as a probe to screen a *Synechococcus* cosmid library. Hybridization of the cosmid library was performed at 65°C. One hybridizing clone was identified and a 2.4-kb *BamHI-NheI* fragment from this cosmid clone was isolated and sequenced.

The 1362-nucleotide DNA segment comprising the *Synechococcus accC* gene is given in SEQ ID NO:7. Only one significant open reading frame (ORF) was found. This ORF potentially encodes a protein of 453 amino acids. The complete translated amino acid sequence of the *Synechococcus accC* gene encoding BC is given in SEQ ID NO:8.

### 5.3.2 Biotin Carboxyl Carrier Protein (*accB*)

In *Synechococcus* PCC 7942, the *accB* gene is not immediately upstream of *accC*, as it is in *E. coli*. Gene-specific DNA probes from both *E. coli* and *Anabaena* PCC7120 *accB* failed to hybridize with the *Synechococcus* genomic DNA by Southern analysis. A different approach was necessary.

Since biotin carboxyl carrier protein is biotinylated and streptavidin has a strong specific affinity for biotin, streptavidin was used to identify the number of biotin-containing proteins in *Synechococcus* PCC 7942. The proteins (from a crude whole protein extract) of *Synechococcus* PCC 7942 were first separated by standard SDS-PAGE method, then transferred onto an Immobilon-P® transfer membrane, which was subsequently incubated with <sup>35</sup>S-streptavidin. Only one radioactive band (corresponding to a protein of about 25 kDa) appeared on the autoradiogram. This result suggests that there is only one biotin-containing protein in *Synechococcus* and its mass is similar to the reported mass of *E. coli* biotin carboxyl carrier protein, 22,500 Da.



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This biotin-containing protein was purified. *Synechococcus* cells were first broken by sonication in a buffer containing NaCl, Tris, glycerol and SDS. The supernatant was separated from cell debris by centrifugation, then followed by a 50%  $(\text{NH}_4)_2\text{SO}_4$  precipitation. The precipitate was dissolved in the same buffer, and was allowed to bind to streptavidin agarose beads. The bound agarose beads were washed and the bound proteins were eluted with 70% formic acid. The formic acid-eluted portion was dried and washed with water before loading onto an acrylamide gel. After electrophoresis, the proteins were transferred from the gel to an Immobilon-P® transfer membrane. The membrane was stained briefly with Coomassie Brilliant blue dye, destained in a mixture of methanol and acetic acid, and soaked in water for an hour or so before air drying. The band corresponding to the streptavidin-bound protein was cut out and its N-terminal amino acid sequence was determined.

Based on the amino acid sequence from the N-terminus of the *Synechococcus* biotin-containing protein and the amino acid sequence around the biotinylation site in all other known BCCPs, degenerate oligonucleotide primers were designed for PCR™ amplification studies with *Synechococcus* genomic DNA. The pair of primers were:

primer LE8 5'-GCTCTAGACNCARYTNAAYTT-3' (SEQ ID NO:26)

primer LE7 3'-CRNTACTTYGACNWCTTAAGCT-5' (SEQ ID NO:27)

PCR™ was performed for 40 cycles (each with 1 minute at 95°C, 1 minute at 48°C, 2 minutes at 72°C), with Cetus *Taq* polymerase, 0.5 mg/ml of template DNA, 5 mg/ml of primer LE8, 40 mg/ml of primer LE7 and with 1 mM  $\text{Mg}^{2+}$  final concentration. Under these conditions, a specific PCR™ product was identified. Sequence analysis of this cloned PCR™ product indicated that it encoded a region of conserved amino acids within *accB* of *Synechococcus* PCC 7942 (compared to the amino acid sequences of the biotin carboxyl carrier protein from *Anabaena* PCC 7120 and *E. coli*). Using this PCR™ fragment as a probe in Southern hybridization, a positive clone was identified from the *Synechococcus* cosmid library. A 1.6-kb *Pst*I fragment from this positive cosmid clone was isolated and sequenced.

A 477-nucleotide DNA segment comprising the *Synechococcus accB* gene is given in SEQ ID NO:3. Only one significant ORF was found. The deduced amino

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acid sequence at the N-terminus of this ORF matches the earlier determined N-terminal amino acid sequence of the purified *Synechococcus* biotin-containing protein. The 158-amino acid sequence of the *Synechococcus* BCCP is given in SEQ ID NO:4. Sequence alignment indicated that the translational product of *accB* from *Synechococcus* PCC 7942 is closer to that from *Anabaena* PCC 7120 than that from *E. coli* (53% versus 31% amino acid identity).

### 5.3.3 Carboxyltransferase $\alpha$ Subunit (CT $\alpha$ , *accA*)

A 0.9-kb *Cla*I-*Mlu*I fragment of the *E. coli accA* gene was used as a probe to examine the *Synechococcus* PCC 7942 genomic DNA by Southern hybridization at 60°C. A strongly hybridizing 1.6-kb *Pst*I fragment was detected and subsequently cloned.

*Synechococcus* PCC 7942 genomic DNA was digested with *Pst*I and electrophoresed on an agarose gel. The gel region containing DNA of sizes between 1.6 and 2.5-kb was cut out and purified. A size library between 1.6-kb and 2.5-kb was created by cloning the purified fraction of *Synechococcus* PCC 7942 DNA into vector pBR322. Tetracycline-resistant, but ampicillin-sensitive, colonies (*i.e.*, with insert) were selected by first plating on LB plates containing tetracycline, then scored on plates containing ampicillin.

A total of 800 tetracycline-resistant, but ampicillin-sensitive, clones were screened: the plasmid DNA was prepared, digested (in pools of 5 clones per pool) with *Pst*I, electrophoresed, transferred onto a Genescreen Plus membrane, then hybridized with the *E. coli accA* probe at 60°C. Positive signals appeared on 3 pools. One positive individual clone, with 2 fragments inserted, was identified at the second round of screening. The positive fragment was isolated and re-cloned. This cloned 1.6-kb *Pst*I fragment was then used as a probe to screen the *Synechococcus* cosmid library where 9 positive clones were identified. A 5-kb *Bam*HI fragment from one of these 9 clones was isolated and sequenced. DNA sequence analysis of the region indicated a cluster of three ORFs in the same orientation.

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The 984-nucleotide DNA segment comprising the *Synechococcus accA* gene is given in SEQ ID NO:11. The first open reading frame encodes the  $\alpha$  subunit of the carboxyltransferase. The 327-amino acid sequence of the *Synechococcus* ORF is 54% identical to that of the *E. coli accA* gene. The amino acid sequence of the

5 *Synechococcus accA* gene encoding CT $\alpha$  is given in SEQ ID NO:12.

#### 5.3.4 Carboxyltransferase $\beta$ Subunit (CT $\beta$ , *accD*)

Oligonucleotide primers, for polymerase chain reaction (PCR<sup>TM</sup>) amplification experiments with *Synechococcus* genomic DNA, were based on the sequence of

10 ORF326 (which is a homolog of the *E. coli accD*) from a different cyanobacterium, *Synechocystis* PCC 6803. The pair of primers were:

LE39 5'-GAAGATCTTTATGGGCGGTAGTATG-3' (SEQ ID NO:28)

LE40 3'-GGTCGAAACGGTACAACCTAGGC-5' (SEQ ID NO:29)

PCR<sup>TM</sup> was run for 40 cycles (each with 1 minute at 95°C, 1 minute at 50°C, 2

15 minutes at 72°C), with Boehringer-Mannheim *Taq* polymerase, 0.5 mg/ml of template DNA, 5 mg/ml of each primer and with 1 mM Mg<sup>2+</sup> final concentration. Under these conditions, a specific PCR<sup>TM</sup> product of 256 bp was identified. Sequence analysis of this cloned PCR<sup>TM</sup> fragment showed a significant similarity between the *Synechococcus* and *Synechocystis* genomic DNAs in the region between the primers.

20 Using this cloned PCR<sup>TM</sup> product as a probe, 5 positive cosmid clones were identified from the *Synechococcus* cosmid library by Southern hybridization.

#### 5.4 EXAMPLE 4 -- Isolation and Characterization of the Wheat ACC Enzyme

25 Biotin-containing (streptavidin-binding) proteins in extracts prepared from leaves of two-week old seedlings of wheat and pea, both total protein and protein from intact chloroplasts (prepared by centrifugation on Percoll gradients as described previously in Fernandez and Lamppa, 1991), and from wheat germ (Sephadex G-100 fraction prepared as described below) were analyzed by western blotting with

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<sup>35</sup>S-Streptavidin. Proteins were separated by SDS-PAGE using a 7.5% separating gel (Maniatis *et al.*, 1982), and then were transferred onto a PVDF membrane (Immobilon-P®, Millipore) in 10 mM 3-(cyclohexylamino)-1-propanesulfonic acid buffer (pH 11), 10% methanol, at 4°C, 40 V, overnight. The blots were blocked with  
5 3% BSA solution in 10 mM Tris-HCl pH 7.5 and 0.9% NaCl and then incubated for 3-16 h with <sup>35</sup>S-Streptavidin (Amersham). The blots were washed at room temperature with 0.5% Nonidet-P40™ in 10 mM Tris-HCl pH 7.5 and 0.9% NaCl.

In wheat, the 220-kDa protein was present in both total and chloroplast protein. It was the major biotinylated polypeptide in the chloroplast protein (traces of  
10 smaller biotinylated polypeptides, most likely degradation products of the large one, could also be detected). ACC consisting of 220-kDa subunits is the most abundant biotin-dependent carboxylase present in wheat chloroplasts. In pea chloroplasts the biotinylated peptides are much smaller, probably due to greater degradation of the 220-kDa peptide, which could be detected only in trace amounts in some chloroplast  
15 preparations. The amount of all biotinylated peptides, estimated from band intensities on western blots (amount of protein loaded was normalized for chlorophyll content), is much higher in pea than in wheat chloroplasts.

Purification of wheat germ ACC was carried out at 4°C or on ice. 200 g of wheat germ (Sigma) were homogenized (10 pulses, 10 s each) in a Waring blender  
20 with 300 ml of 100 mM Tris-HCl pH 7.5, 7 mM 2-mercaptoethanol. Two 0.3 ml aliquots of fresh 0.2 M solution of phenylmethyl-sulfonyl fluoride (Sigma) in 100% ethanol were added immediately before and after homogenization. Soluble protein was recovered by centrifugation for 30 min at 12000 rpm. 1/33 volume of 10% poly(ethyleneimine) solution (pH 7.5) was added slowly and the mixture was stirred  
25 for 30 min (Egin-Buhler *et al.*, 1980), followed by centrifugation for 30 min at 12000 RPM to remove the precipitate. ACC in the supernatant was precipitated by adding solid ammonium sulfate to 50% saturation.

The precipitate was collected by centrifugation for 30 min at 12000 rpm, dissolved in 200 ml of 100 mM KCl, 20 mM Tris-HCl pH 7.5, 20% glycerol, 7 mM  
30 2-mercaptoethanol, mixed with 0.2 ml of phenylmethysulfonyl fluoride solution (as

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above) and loaded on a 5 cm × 50 cm Sephadex G-100 column equilibrated and eluted with the same buffer. Fractions containing ACC activity (assayed as described below using up to 20 µl aliquots of column fractions) were pooled and loaded immediately on a 2.5 cm × 40 cm DEAE-cellulose column also equilibrated with the same buffer.

5 The column was washed with 500, 250 and 250 ml of the same buffer containing 150, 200 and 250 mM KCl, respectively. Most of the ACC activity was eluted in the last wash. Protein present in this fraction was precipitated with ammonium sulfate (50% saturation), dissolved in a small volume of 100 mM KCl, 20 mM Tris-HCl pH 7.5, 5% glycerol, 7 mM 2-mercaptoethanol, and separated in several portions on two  
10 Superose columns connected in-line (Superose 6 and 12, Pharmacia). 1 ml fractions were collected at 0.4 ml/min flow rate. Molecular mass standards were thyroglobulin, 669-kDa; ferritin, 440-kDa; aldolase, 158-kDa; albumin, 67-kDa (Pharmacia). ACC-containing fractions were concentrated using Centricon-100 concentrators (Amicon) and the proteins were separated by SDS-PAGE as described above.

15 By gel filtration, active ACC had an apparent molecular mass of ~ 500-kDa and the individual polypeptides have a molecular mass of 220-kDa. The 220-kDa polypeptide was the major component of this preparation as revealed by Coomassie staining of proteins separated by SDS-PAGE. This preparation also contained several smaller biotin-containing peptides as revealed by western blotting with  
20 <sup>35</sup>S-Streptavidin, most likely degradation products of the ca. 220-kDa peptide, which retained their ability to form the ~500-kDa complex and therefore co-purified with intact ACC. The ACC preparations were active only when they contained intact 220-kDa biotinylated polypeptide. It is not possible to estimate the recovery of the active ACC, due to continuous degradation of the 220-kDa peptide during purification and to  
25 increased recovery of ACC activity in more purified preparations, probably due to separation of the enzyme from inhibitors in the cruder extracts.

The 220-kDa wheat peptide isolated as a dimer according to the above protocol was finally purified by SDS-PAGE and transferred to Immobilon-P® for sequencing. The N-terminus of the peptide appeared to be blocked. A mixture of  
30 amino acids was detected only after the protein was cleaved chemically with CNBr.

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The 220-kDa protein was therefore purified on an SDS gel, cleaved with CNBr, and the resulting peptides were fractionated by gel electrophoresis basically as described (Jahnen-Dechent and Simpson, 1990), with the following modifications. A slice of gel containing about 20 µg of the 220-kDa polypeptide was dried under vacuum to about half of its original volume and then incubated overnight in 0.5 ml of 70% formic acid containing 25 mg of CNBr. The gel slice was dried again under vacuum to about half of its original volume and was equilibrated in 1 ml of 1 M Tris-HCl (pH 8.0). The CNBr peptides were separated by inserting the gel piece directly into a well of a tricine gel (as described in Jahnen-Dechent and Simpson, 1990; but without a spacer gel). Gels used to separate peptides for sequencing were pre-run for 30 min with 0.1 mM thioglycolic acid in the cathode buffer. Peptides were transferred to Immobilon-P for sequencing by the Edman degradation method as described above.

Several bands of peptides, ranging in size from 4 to 16-kDa, with a well-resolved single band at about 14-kDa, were obtained. Attempts to sequence the smaller peptides failed, but the 14-kDa peptide yielded a clean results for residues 3-13.

### 5.5 EXAMPLE 5 -- Effects of the Herbicide Haloxyfop on Wheat ACC

The effect of haloxyfop, one of the aryloxyphenoxypropionate herbicides has been tested, on the activity of ACC from wheat germ and from wheat seedling leaves.

For the *in vitro* assay of ACC activity, 1-8 µl aliquots of ACC preparations were incubated for 45 min at 37°C with 20 µl of 100-200 mM KCl, 200 mM Tris-HCl pH 8.0, 10 mM MgCl<sub>2</sub>, 2 mM ATP, 2 mM DTT, 2 mM <sup>14</sup>C-NaHCO<sub>3</sub>, and where indicated 1 mM Ac-CoA, in a final volume of 40 µl. The reaction was stopped by adding 4 µl of concentrated HCl 30-40 µl aliquots of the reaction mixture were spotted on filter paper and dried, and acid-stable radioactivity was measured using scintillation cocktail. Haloxyfop was added as the Tris salt of the acid, generously supplied by J. Secor of Dow-Elanco.

For the *in vivo* assay of ACC activity, 2-week old seedlings of wheat (*Triticum aestivum* cv. Era) were cut about 1 cm below the first leaf and transferred to a 1.5 ml

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micro tube containing  $^{14}\text{C}$ -sodium acetate and haloxyfop (Tris salt) for 4-6 h. The leaves were then cut into small pieces and treated with 0.5 ml of 40% KOH for 1 h at 70°C, and then with 0.3 ml of  $\text{H}_2\text{SO}_4$  and 20  $\mu\text{l}$  of 30% TCA on ice. Fatty acids were extracted with three 0.5 ml aliquots of petroleum ether. The organic phase was washed with 1 ml of water. Incorporation of  $^{14}\text{C}$ -acetate into fatty acids is expressed as the percentage of the total radioactivity taken up by the seedlings, present in the organic phase.

As expected, the enzyme from wheat germ or from wheat chloroplasts was sensitive to the herbicide at very low levels. 50% inhibition occurs at about 5 and 2  $\mu\text{M}$  haloxyfop, respectively. For comparison, the enzyme from pea chloroplasts is relatively resistant (50% inhibition occurs at  $>50$  :M haloxyfop). Finally, the *in vivo* incorporation of  $^{14}\text{C}$ -acetate into fatty acids in freshly cut wheat seedling leaves is even more sensitive to the herbicide (50% inhibition occurs at  $<1$  :M haloxyfop), which provides a convenient assay for both ACC and haloxyfop.

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## 5.6 EXAMPLE 6 -- Cloning and Sequencing of *Triticum aestivum* ACC cDNA

### 5.6.1 Materials and Methods

#### 5.6.1.1 PCR™ Amplification

Degenerate PCR™ primers were based on the alignment of amino acid sequences of the following proteins (accession numbers in brackets): rat (J03808) and chicken (J03541) ACCs; *E. coli* (M80458, M79446, X14825, M32214), *Anabaena* 7120 (L14862, L14863) and *Synechococcus* 7942 BCs and BCCPs; rat (M22631) and human (X14608) propionyl-coenzyme A carboxylase (" subunit); yeast (J03889) pyruvate carboxylase; *Propionibacterium shermanii* (M11738) transcarboxylase (1.3S subunit) and *Klebsiella pneumonia* (J03885) oxaloacetate decarboxylase (a subunit). Each primer consisted of a 14-nucleotide specific sequence based on the amino acid sequence and a 6- or 8-nucleotide extension at the 5'-end.

Poly(A)<sup>+</sup> RNA from 8-day old plants (*Triticum aestivum* var. Era) was used for the synthesis of the first strand of cDNA with random hexamers as primers for AMV reverse transcriptase (Haymerle *et al.*, 1986). Reverse transcriptase was

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inactivated by incubation at 90°C and low molecular weight material was removed by filtration. All components of the PCR™ (Cetus/Perkin-Elmer), except the *Taq* DNA polymerase, were incubated for 3-5 min at 95°C. The PCR™ was initiated by the addition of polymerase. Conditions were optimized by amplification of the BC gene from *Anabaena* 7120. Amplification was for 45 cycles, each 1 min at 95°C, 1 min at 42-46°C and 2 min at 72°C. MgCl<sub>2</sub> concentration was 1.5 mM. Both the reactions using *Anabaena* DNA and the single-stranded wheat cDNA as template yielded the expected 440-bp products. The wheat product was separated by electrophoresis on LMP-agarose and reamplified using the same primers and a piece of the LMP-agarose slice as a source of the template. That product, also 440-bp, was cloned into the Invitrogen vector pCR1000 using their A/T tail method, and sequenced.

In eukaryotic ACCs, the BCCP domain is located about 300 amino acids downstream from the end of the BC domain. Therefore, it was possible to amplify the cDNA encoding that interval between the two domains using primers, one from the C-terminal end of the BC domain and the other from the conserved biotinylation site. The expected 1.1-kb product of the first low yield PCR™ with primers III and IV was separated by electrophoresis on LMP-agarose and reamplified by another round of PCR™, then cloned into the Invitrogen vector pCRII® and sequenced. The PCR™ conditions were the same as those described above.

#### 5.6.1.2 Isolation and Analysis of ACC cDNA

A wheat cDNA library (*Triticum aestivum*, var. Tam 107, Hard Red Winter, 13-day light grown seedlings) was purchased from Clontech. This 8gt11 library was prepared using both oligo(dT) and random primers. Colony ScreenPlus® (DuPont) membrane was used according to the manufacturers' protocol (hybridization at 65°C in 1 M NaCl and 10% dextran sulfate). The library was first screened with the 1.1-kb PCR™-amplified fragment of ACC-specific cDNA. Fragments of clones 39-1, 45-1 and 24-3 were used in subsequent rounds of screening. In each case,  $\sim 2.5 \times 10^6$  plaques were tested. More than fifty clones containing ACC-specific cDNA



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fragments were purified, and *EcoRI* fragments of the longest cDNA inserts were subcloned into pBluescriptSK® for further analysis and sequencing. A subset of the clones was sequenced on both strands by the dideoxy chain termination method with Sequenase® (United States Biochemicals) or using the Perkin Elmer/Applied Biosystems Taq DyeDeoxy Terminator cycle sequencing kit and an Applied Biosystems 373A DNA Sequencer.

### 5.6.1.3 RNA and DNA

Total RNA from 10-day old wheat plants was prepared as described in (Haymerle *et al.*, 1986). RNA was separated on a glyoxal denaturing gel (Sambrook *et al.*, 1989). GeneScreen Plus® (DuPont) blots were hybridized in 1M NaCl and 10% dextran sulfate at 65°C (wheat RNA and DNA) or 58-60°C (soybean and canola DNA). All cloning, DNA manipulation and gel electrophoresis were as described (Sambrook *et al.*, 1989).

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## 5.6.2 Results

### 5.6.2.1 PCR™ Cloning of the Wheat (*Triticum aestivum*) ACC cDNA

A 440-bp cDNA fragment encoding a part of the biotin carboxylase domain of wheat ACC and a 1.1-kb cDNA fragment encoding the interval between the biotin carboxylase domain and the conserved biotinylation site were amplified. These fragments were cloned and sequenced. In fact, three different 1.1-kb products, corresponding to closely related sequences that differ from each other by 1.5%, were identified. The three products most likely represent transcription products of three different genes, the minimum number expected for hexaploid wheat. These two overlapping DNA fragments (total length of 1473 nucleotides) were used to screen a wheat cDNA library.

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### 5.6.2.2 Isolation and Sequence Analysis of Wheat ACC cDNAs

A set of overlapping cDNA clones covering the entire ACC coding sequence was isolated and a subset of these clones has been sequenced. The nucleotide

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sequence within overlapped regions of clones 39-1, 20-1 and 45-1 differ at 1.1% of the nucleotides within the total of 2.3 kb of the overlaps. The sequence within the overlap of clones 45-1 and 24-3 is identical. The sequence contains a 2257-amino acid reading frame encoding a protein with a calculated molecular mass of 251 kDa.

5 In wheat germ the active ACC has an apparent molecular mass of ~500 kDa and the individual polypeptides have an apparent molecular mass (measured by SDS-PAGE) of about 220 kDa (Gornicki and Haselkorn, 1993). The 220-kDa protein was also present in both total leaf protein and protein from intact chloroplasts. In fact, it was the major biotinylated polypeptide in the chloroplast protein. The cDNAs (total  
10 length 7.4 kb) include 158 bp of the 5'-untranslated and 427 bp of the 3'-untranslated sequence.

The 7360-nucleotide DNA segment comprising the wheat ACC cDNA is given in SEQ ID NO:9. The 2257-amino acid translated wheat ACC sequence is given in SEQ ID NO:10.

15

#### 5.6.2.3 Northern Analysis of ACC mRNA

Northern blots with total RNA from 10-14 day old wheat leaves were probed using different cDNA fragments (the 1.1-kb PCR<sup>TM</sup>-amplified fragment and parts of clones 20-1, 24-3 and 01-4). In each case the only hybridizing mRNA species was 7.9  
20 kb in size. This result shows clearly that all the cDNA clones correspond to mRNA of large, eukaryotic ACC and that there are no other closely related biotin-dependent carboxylases, consisting of small subunits that are encoded by smaller mRNAs, in wheat.

Northern analysis of total RNA prepared from different sectors of 10-day old  
25 wheat seedlings indicates very high steady-state levels of ACC-specific mRNA in cells of leaf sectors I and II near the basal meristem. The ACC mRNA level is significantly higher in sectors I and II than in sectors III-VI. This cannot be explained by dilution of specific mRNA by increased levels of total RNA in older cells. Based on published results (Dean and Leech, 1982), the increase in total RNA between  
30 sectors I and VI is expected to be only about two-fold.

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All cell division occurs in the basal meristem and cells in other sectors are in different stages of development. Differences between these young cells and the mature cells at the tip of the leaf include cell size, number of chloroplasts and amount of total RNA and protein per cell (Dean and Leech, 1982). Expression of some genes is correlated with the cell age (e.g., Lampa *et al.*, 1985). It is not surprising that the level of ACC-specific mRNA is highest in dividing cells and in cells with increasing number of chloroplasts. The burst of ACC mRNA synthesis is necessary to supply enough ACC to meet the demand for malonyl-coenzyme A. The levels of ACC mRNA decrease significantly in older cells where the demand is much lower. The same differences in the level of ACC specific mRNA between cells in different sectors were found in plants grown in the dark and in plants illuminated for one day at the end of the dark period.

#### 5.6.2.4 Southern Analysis of Plant DNA

Hybridization, under stringent conditions, of wheat total DNA digests with wheat ACC cDNA probes revealed multiple bands. This was expected due to the hexaploid nature of wheat (*Triticum aestivum*). Some of the wheat cDNA probes also hybridize with ACC-specific DNA from other plants. The specificity of this hybridization was demonstrated by sequencing several fragments of canola genomic DNA isolated from a library using wheat cDNA probe 20-1 and by Northern blot of total canola RNA using one of the canola genomic clones as a probe. The Northern analysis revealed a large ACC-specific message in canola RNA similar in size to that found in wheat.

#### 5.6.2.5 ACC mRNA

The putative translation start codon was assigned to the first methionine of the open reading frame. An in-frame stop codon is present 21 nucleotides up-stream from this AUG. The nucleotide sequence around this AUG fits quite well with the consensus for a monocot translation initiation site derived from the sequence of 93 genes, except for U at position +4 of the consensus which was found in only 3 of the

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93 sequences. The ACC mRNA stop codon UGA is also the most frequently used stop codon found in monocot genes, and the surrounding sequence fits the consensus well.

#### 5 5.6.2.6 Homologies with Other Carboxylases

A comparison of the wheat ACC amino acid sequence with other ACCs shows sequence conservation among these carboxylases. The sequence of the polypeptide predicted from the cDNA described above was compared with the amino acid sequences of other ACCs, and about 40% identity are with the ACC of rat, diatom and yeast (about 40%). Less extensive similarities are evident with subunits of bacterial ACCs. The amino acid sequence of the most highly conserved domain, corresponding to the biotin carboxylases of prokaryotes, is about 50% identical to the ACC of yeast, chicken, rat and diatom, but only about 27% identical to the biotin carboxylases of *E. coli* and *Anabaena* 7120. The biotin attachment site has the typical sequence of eukaryotic ACCs. Several conserved amino acids found in the carboxyltransferase domains previously identified (Li and Cronan, 1992) are also present in the wheat sequence. Surprisingly, none of the four conserved motifs containing serine residues, which correspond to phosphorylation sites in rat, chicken and human ACCs (Ha *et al.*, 1994), is present at a similar position in the wheat polypeptide.

#### 5.6.2.7 Lack of Targeting Sequence in Wheat ACC cDNA

The wheat cDNA does not encode an obvious chloroplast targeting sequence unless this is an extremely short peptide. There are only 12 amino acids preceding the first conserved amino acid found in all eukaryotic ACCs (a serine residue). The conserved core of the BC domain begins about 20 amino acids further down-stream. The apparent lack of a transit peptide poses the question of whether and how the ACC described in this paper is transported into chloroplasts. It was shown recently that the large ACC polypeptide purifies with chloroplasts of wheat and maize (Gornicki and Haselkorn, 1993; Egli *et al.*, 1993). No obvious chloroplast transit peptide between

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the ER signal peptide and the mature protein was found in diatom ACC either (Roessler and Ohlrogge, 1993).

The number of ACC genes in wheat have been assessed by Southern analysis and by sequence analysis of the 5'- and 3'-untranslated portions of ACC cDNA representing transcripts of different genes. These cDNA fragments may be obtained by PCR™ amplification using the 5'- and 3'-RACE methodology. The genome structure of wheat (*Triticum aestivum*) suggests the presence of at least three copies of the ACC gene, i.e. one in each ancestral genome. Sequence analysis of the 5'-untranscribed parts of the gene may determine whether any familiar promoter and regulatory elements are present. The structure of introns within the control region and in the 5'-fragment of the coding sequence is also of interest.

The plant ACC genes are full of introns and their transcripts undergo alternative splicing. In some plant genes, introns have been found both within the sequence encoding the transit peptide, and at the junction between the transit peptide and the mature protein.

In plants, variant cytoplasmic and plastid isoenzymes could arise, for example, by alternative splicing or by transcription of two independent genes. This problem is especially intriguing as it was not possible to identify a transit peptide in the sequences of wheat ACC obtained so far. The two possibilities can be distinguished by sequence analysis of the appropriate fragment of the ACC genes (clones from genomic library) and mRNAs (as cDNA). The sequence of these 5'- and 3'-untranscribed and untranslated fragments of the gene are usually significantly different for different alleles so they may also be used as specific probes to follow expression of individual genes.

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#### 5.7 EXAMPLE 7 -- DNA Compositions Comprising a Wheat Cytosolic ACC

This example describes the cloning and DNA sequence of the entire gene encoding wheat (var. Hard Red Winter Tam 107) acetyl-CoA carboxylase (ACCase). Comparison of the 12-kb genomic sequence (SEQ ID NO:30) with the 7.4-kb cDNA sequence reported in Example 6 revealed 29 introns. Within the coding region (SEQ

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ID NO:31), the exon sequence is 98% identical to the wheat cDNA sequence (SEQ ID NO:XX). A second ACCase gene was identified by sequencing fragments of genomic clones that include the first two exons and the first intron. Additional transcripts were detected by 5'- and 3'-RACE analysis. One set of transcripts had 5'-end sequence  
5 identical to the cDNA found previously and another set was identical to the gene reported here. The 3'-RACE clones fall into four distinguishable sequence sets, bringing the number of ACCase sequences to six. None of these cDNA or genomic clones encode a chloroplast targeting signal. Identification of six different sequences suggests that either the cytosolic ACCase genes are duplicated in the three  
10 chromosome sets in hexaploid wheat or that each of the six alleles of the cytosolic ACCase gene has a readily distinguishable DNA sequence.

### 5.7.1 Materials and Methods

#### 5.7.1.1 Isolation and Analysis of ACCase Genomic Clones

15 A wheat genomic library (*T. aestivum*, var. Hard Red Winter Tam 107, 13-day light grown seedlings) was purchased from Clontech. This 8 EMBO3 library was prepared from genomic DNA partially digested with *Sau3A*. Colony ScreenPlus (DuPont) membrane was used according to the manufacturers' protocol (hybridization at 65°C in 1M NaCl and 10% dextran sulfate). The library was screened with a  
20 440-bp PCR<sup>TM</sup>-amplified fragment of ACCase-specific cDNA and with cDNA clone 24-3 (Gornicki *et al.*, 1994). In each case,  $\sim 1.2 \times 10^6$  plaques were tested. 24 clones containing ACCase-specific DNA fragments were purified and mapped. Selected restriction fragments of these genomic clones were subcloned into pBluescriptSK<sup>®</sup> for further analysis and sequencing. The 3'-terminal fragment of the gene (clone 145)  
25 was amplified by PCR<sup>TM</sup> using wheat genomic DNA as a template. Primers were based on the sequence of genomic clone 233, 5'-CGCTATAGGGAAACGTTAGAAGGATGGG-3' (SEQ ID NO:34) and 3'-RACE clone 4, 5'-ATCGATCGGCCTCGGCTCCAATTTTCATT-3' (SEQ ID NO:35).

All PCR<sup>TM</sup> components except *Taq* polymerase were incubated for 5 min. at  
30 95°C. The reactions were initiated by the addition of the polymerase followed by 35

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cycles of incubation at 94°C for 1min, 55°C for 2 min and 72°C for 2 min. A 1.8-kb PCR™ product was gel-purified, reamplified using the same primers, cloned into the Invitrogen vector pCRII™ and sequenced.

5    5.7.1.2 Analysis of mRNA by rapid amplification of cDNA ends (RACE)

Two sets of 15 and 20 cDNA fragments corresponding to mRNA 5'- and 3'-ends, respectively, were prepared by T/A cloning of RACE products into the vector pCRII. Total RNA from 15-day old wheat (*Triticum aestivum* var. Tam 107, Hard Red Winter) plants was prepared as described in Chirgwin *et al.* (1979). A Gibco  
10    BRL 5'-RACE kit was used according to the manufacturers' protocol. For the 5'-end amplification, the first strand of cDNA was prepared using a gene-specific primer: 5'-GTTCCCAAAGGTCTCCAAGG-3' (SEQ ID NO:36); followed by the addition of a homopolymeric dA-tail.

dT-Anchor primer: 5'-GCGGACTCGAGTCGACAAGCTTTTTTTTTTTTTTTTTT-3'  
15    (SEQ ID NO:37); and a gene-specific primer, 5'-ACGCGTCGACTAGTAGGTGCGGATGCTGCGCATG-3' (SEQ ID NO:38) were used in the first round of PCR™.

Universal primer, 5'-GCGGACTCGAGTCGACAAGC-3' (SEQ ID NO:39) and another gene-specific primer, 5'-ACGCGTCGACCATCCCA  
20    TTGTTGGCAACC-3' (SEQ ID NO:40) were used for reamplification. The gene-specific primers were targeted to a stretch of 5'-end coding sequence identical in clones 39 and 71 that were available.

Clone 71 was isolated from a 8gt11 cDNA library as described before using a fragment of cDNA 39 as probe (Example 4). The same dT-anchor primer and  
25    universal primer together with a gene specific primer 5'-GACTCATTGAGATCAAGTTC-3' (SEQ ID NO:41) were used for the first strand cDNA synthesis and 3'-end amplification. The latter primer was targeted to the 3'-end of the ACCase open reading frame.

All cloning, DNA manipulations and gel electrophoresis were as described  
30    (Sambrook *et al.*, 1989). DNA was sequenced on both strands by the dideoxy chain

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termination method using  $^{35}\text{S}$ -[dATP] with Sequenase (United States Biochemicals) or using the Perkin Elmer/Applied Biosystems Taq DyeDeoxy Terminator cycle sequencing kit and an Applied Biosystems 373A DNA Sequencer.

## 5 5.7.2 Results

### 5.7.2.1 Analysis of wheat cytosolic ACCase genes

Two cDNA fragments, one encoding a part of the biotin carboxylase domain of wheat ACCase and the other a part of the carboxyltransferase, were used to isolate a set of overlapping DNA fragments covering the entire ACCase gene. Some of these genomic fragments were sequenced as indicated in FIG. 1. Where they overlap, the nucleotide sequences of clones 31, 191 and 233 are identical. These obviously derive from the same gene. cDNA clone 71 (see below) represents the transcription product of this gene (430-nucleotide identical sequence). The sequence of clone 145 obtained by PCR<sup>TM</sup> to cover the remaining 3'-end part of the gene differs from clone 233 by 5 of 400 nucleotides of the overlap located within the long exon 28 (FIG. 1). It must therefore derive from a different copy of the ACCase gene. 3'-RACE clone 4 (3'-4, see below) differs at 6 of 490 nucleotides in the overlap.

The sequence was deposited in GenBank (as accession number U39321), and is a composite of these three very closely related sequences. Its 5'-end corresponds to the 5'-end of clone 71 and the 3'-end corresponds to the poly(A) attachment site of the 3'-RACE clone 4. It was assumed that no additional introns are present at the very end of the gene.

Comparison of the genomic sequence with the cDNA sequence in Example 4 revealed 29 introns. Intron location is conserved among all three known plant ACCase genes except for two introns not present in wheat but found in rape (Schulte *et al.*, 1994), *A. thaliana* (Roesler *et al.*, 1994) and soybean (Anderson *et al.*, 1995) (FIG. 1). The nucleotide sequence at splice sites fits well with the consensus for monocot plants. The A+T content of the gene exons and introns is 52% and 63%, respectively, compared to 42% and 61% found for other monocot plant genes (White *et al.*, 1992). The exon coding sequence is 98% identical to that of the cDNA



sequence reported earlier. This is the same degree of identity as found previously for different transcripts of the cytosolic ACCase genes in hexaploid wheat (Example 4). The 11-amino acid sequence obtained previously for a CNBr-generated internal fragment of purified 220-kDa wheat germ ACCase (Gornicki and Haselkorn, 1993) differs from the sequence encoded by these cDNA and genomic clones at one position, but it is identical with the corresponding cDNA sequence of the plastid ACCase from maize (Egli *et al.*, 1995), excluding one amino acid which could not be assigned unambiguously in the sequence.

Two additional genomic clones, 153 and 231, were also partially sequenced (FIG. 1). The sequenced fragments include parts of the first two exons and the first intron. Although cDNA corresponding exactly to genomic clone 153 is not available, the boundaries of the first intron could easily be identified by sequence comparison with cDNA clone 71 (corresponding to genomic clone 31). Clone 153 encodes a polypeptide that differs by only one out of the first 110 amino acids of the ACCase open reading frame. The sequence of the 5'-leader was also well conserved but the 5'-part of the first intron of clone 153 is significantly different from that of genomic clone 31.

On the other hand, only the 3'-splice site of an intron could be identified by sequence comparison in this part of clone 231. The sequence immediately upstream of the 3'-splice site and that of the following exon is identical to that of clone 31. No sequence related to that found upstream of the first intron of clone 191 could be identified in clone 231 by hybridization (including a ~6 kb fragment upstream of the ACCase open reading frame) or by sequencing (~ 2 kb of the upstream fragment). It is possible that the first intron in this gene is much larger (additional upstream introns can not be excluded) or that the upstream exon(s) and untranscribed part of the gene has a completely different sequence. A cloning artifact can not be ruled out. Indeed clone 31 contained such an unrelated sequence at its 5'-end (probably a ligation artifact).

Identification of three additional genomic clones with sequence closely related to the other ACCase genes but containing no introns at several tested locations

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suggests the existence of a pseudogene in wheat. A fragment of clone 232 that was sequenced is represented in the diagram shown in FIG. 1. It is 93% and 96% identical with clone 233 at the nucleotide and amino acid level, respectively.

Shown in FIG. 5 is the 5' flanking sequence of the ACCase 1 gene (about 3 kb upstream of the translation initiation codon, of clone 71L (SEQ ID NO:32). The 5' flanking sequence of the ACCase 2 gene designated 153 (SEQ ID NO:33) is shown in FIG. 6.

#### 5.7.2.2 Analysis of mRNA ends

In the original library screen (Gornicki *et al.*, 1994) it was not possible to isolate any cDNA clones corresponding to the very ends of the ACCase mRNA. With the new sequence available it became possible to generate the missing pieces by RACE. Two sets of 5'-end RACE clones, 71L and 39L, were identified. Their sequence is identical to the sequence of cDNA clones 71 (this work) and 39 (Gornicki *et al.*, 1994), respectively. The two sequences extend 239 and 312 nucleotides upstream of the ACCase initiation codon and define an approximate position of the transcription start site. None of the genomic clones corresponds to 39L. The presence of the first intron in the corresponding gene could not therefore be confirmed. All three coding sequences are very similar (they differ by only one three-amino acid deletion or one E to D substitution found within the first 110 amino acids) and none of them encodes additional amino acids at the N-terminus, *i.e.*, none of them encodes a potential chloroplast transit peptide.

The sequences of the 5'-leaders differ significantly although they share some distinctive structural features. They are relatively long (at least 239-312 nucleotides as indicated by the lengths of 39L and 71L, respectively), G+C rich (67%) and contain upstream AUG codons. The open reading frames found in the leaders are 70-90 amino acids long and they end within a few nucleotides of the ACCase initiation codon. A similar arrangement was found in the sequence of genomic clone 153. The three upstream AUG codons are conserved and the presence of deletions, most of which are a multiple of three nucleotides, suggests at least some conservation of the

open reading frames at the amino acid level. This arrangement, found in the cytosolic ACCase genes, contrasts with the majority of 5'-untranslated leaders found in plants. Although much longer leader sequences containing upstream AUG codons have been reported in plants (*e.g.*, Shorrosh *et al.*, 1995), they are rare. In most cases, the first  
5 AUG codon is the site of initiation of translation of the major gene product. The upstream AUGs are believed to affect the efficiency of mRNA translation and as such may be important in the regulation of expression of some genes (Roesler *et al.*, 1994; Anderson *et al.*, 1995). They are often found in mRNAs encoding transcription factors, growth factors and receptors, all important regulatory proteins (Kozak, 1991).  
10 They are also found in some plant mRNAs encoding heat shock proteins (Joshi and Nguyen, 1995). The ~800 nucleotide long leader intron found in both genes (clones 153 and 191) may also be important for the level and pattern of gene expression (*e.g.*, Fu *et al.*, 1995).

Four different sequences and two different polyadenylation sites ~300 and  
15 ~500 nucleotides downstream of the translation stop codon, respectively, were detected among the 3'-end RACE clones (FIG. 2). The sequence of the cDNA reported previously (Gornicki *et al.*, 1994) and the sequence of genomic clone 145 are also different in this region, bringing the total number of different sequences to six. 3-14 nucleotide differences were found in pairwise comparisons among these six  
20 sequences within two stretches that include 282 nucleotides at the 5'-end of the 3'-RACE clones and 204 nucleotides at the 3'-end (FIG. 2).

### 5.7.2.3 Cytosolic ACC

A gene encoding eukaryotic-type cytosolic ACCase from wheat, very similar  
25 in sequence to the cDNA in Example 4, was cloned and sequenced. Nucleotide identity between the cDNA and the gene within the coding sequence is 98%. The putative translation start codon was assigned in the original cDNA sequence to the first methionine of the open reading frame. An in-frame stop codon is present 21 nucleotides upstream from this AUG and the conserved core of the biotin carboxylase  
30 domain begins about 20 amino acids further down-stream. The gene, shown in FIG. 3

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(SEQ ID NO:30), encodes a 2260-amino acid protein with a calculated molecular mass of 252 kDa (FIG. 4 and SEQ ID NO:31). The wheat cDNA did not encode an obvious chloroplast targeting sequence. The same is true for all the cDNA and genomic sequences described in this paper. The cDNA for maize plastid ACCase, reported recently (Egli *et al.*, 1995), does encode a chloroplast transit peptide.

Comparison of the ACCase sequence encoded by the gene reported in this paper with the sequence of the wheat ACCase of Example 4 and with other representative biotin-dependent carboxylases is shown in Table 4. Wheat ACCase is most similar to other eukaryotic-type plant ACCases. Identity with other eukaryotic carboxylases is also significant. The core sequence of the most conserved ACCase domain, biotin

**TABLE 4**  
**Amino Acid Identities (%) Between *T. aestivum* Cytosolic ACCase**  
**and Some Other Representative Biotin-Dependent Carboxylases**

Specimen	Location	Full Length	Biotin Carboxylase Domain	References
<i>Eukaryotic type carboxylases</i>				
<i>T. aestivum</i> <sup>1</sup>	cytosolic	99	99	Gornicki <i>et al.</i> , 1994
<i>A. thaliana</i>	cytosolic	72	87	Roesler <i>et al.</i> , 1994
<i>M. sativa</i>	cytosolic	73	86	Shorosh <i>et al.</i> , 1994
<i>B. napus</i> <sup>2</sup>		68	82	Schulte <i>et al.</i> , 1994
<i>Z. mays</i>	plastid	71	81	Egli <i>et al.</i> , 1995
<i>R. ratus</i>	cytosolic	40	59	Lopez-Casillas <i>et al.</i> , 1988
<i>C. cryptica</i> <sup>2</sup>		38	55	Roessler and Ohlrogge, 1993
<i>S. cerevisiae</i>	cytosolic	40	56	Al-Feel <i>et al.</i> , 1992
<i>Prokaryotic type carboxylases</i>				
<i>E. coli</i> <sup>3</sup>	bacterial	-	33	Li and Cronan, 1992
<i>Anabaena</i> 7120 <sup>3</sup>	bacterial	-	34	Gornicki <i>et al.</i> , 1993

Specimen	Location	Full Length	Biotin Carboxylase Domain	References
<i>M. leprae</i> <sup>4</sup>	bacterial	-	32	Norman <i>et al.</i> , 1994
<i>N. tabacum</i> <sup>3</sup>	plastid	-	32	Shorosh <i>et al.</i> , 1995
<i>R. ratus</i> PCC <sup>5</sup>	mitochondrial	-	34	Browner <i>et al.</i> , 1989
<i>S. cerevisiae</i> PC <sup>6</sup>	mitochondrial	-	32	Lim <i>et al.</i> , 1988
<i>A. thaliana</i> MCCase <sup>7</sup>	mitochondrial	-	34	Weaver <i>et al.</i> , 1995

<sup>1</sup>Sequence deduced from cDNA sequence reported previously (product of a different allele or gene).

<sup>2</sup>Cellular localization uncertain.

<sup>3</sup>Biotin carboxylase subunit of ACCase.

<sup>4</sup>Biotin carboxylase-biotin carboxyl carrier subunit of ACCase.

<sup>5</sup>Biotin carboxylase-biotin carboxyl carrier subunit (a) of propionyl-CoA carboxylase.

<sup>6</sup>Pyruvate carboxylase.

<sup>7</sup>Biotin carboxylase-biotin carboxyl carrier subunit of methylcrotonyl-CoA carboxylase.

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carboxylase, is well conserved in both eukaryotic and prokaryotic biotin-dependent carboxylases. The other functional domains are less conserved (Example 4). Among plant eukaryotic-type ACCases, the wheat cytosolic ACCase is no more similar to the  
5 maize plastid ACCase (both monocots) than it is to cytosolic ACCases from dicot plants. Clearly, cytosolic and plastid eukaryotic-type ACCases are quite distinct proteins. Another wheat ACCase for which partial sequence is available (Elborough *et al.*, 1994) is most likely a plastid isozyme. It is more similar to the maize plastid ACCase than to the wheat cytosolic enzyme. The plant prokaryotic-type plastid  
10 enzyme is more similar to bacterial, most notably cyanobacterial ACCases and to biotin-dependent carboxylases found in mitochondria, than to any of the plant cytosolic ACCases.

Sequence comparison of fragments of cDNA and genomic clones from the 3Nend of the gene brings the total number of different genes encoding cytosolic  
15 ACCase in wheat to six, indicating that in hexaploid wheat there are at least two distinguishable coding sequences for the cytosolic ACCase in each of the three ancestral chromosome sets. Those two sequences might correspond to the alleles of the ACCase gene present in each ancestral chromosome set. On the other hand, it is possible that each pair of alleles has identical sequences, since the bread wheat studied  
20 is extensively inbred. If that is the case, then one or more ancestral genes has been duplicated.

### 5.8 EXAMPLE 8 -- Developmental Analysis of ACC Genes

Methods have been developed for analyzing the regulation of ACC gene  
25 expression on several levels. With the cDNA clones in hand, the first may be obtained by preparing total RNA from various tissues at different developmental stages *e.g.*, from different segments of young wheat plants, then probing Northern blots to determine the steady-state level of ACC mRNA in each case. cDNA probes encoding conserved fragments of ACC may be used to measure total ACC mRNA  
30 level and gene specific probes to determine which gene is functioning in which tissue.

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In parallel, the steady-state level of ACC protein (by western analysis using ACC-specific antibodies and/or using labeled streptavidin to detect biotinylated peptides) and its enzymatic activity may be measured to identify the most important stages of synthesis and reveal mechanisms involved in its regulation. One such study  
5 evaluates ACC expression in fast growing leaves (from seedlings at different age to mature plants), in the presence and in the absence of light.

### 5.9 EXAMPLE 9 -- Isolation of Herbicide-Resistant Mutants

Development of herbicide-resistant plants is an important aspect of the present  
10 invention. The availability of the wheat cDNA sequence facilitates such a process. By insertion of the complete ACC cDNA sequence into a suitable yeast vector in place of the yeast ACC coding region, it is possible to complement a FAS3 mutation in yeast using procedures well-known to those of skill in the art (see *e.g.*, Haslacher  
15 *et al.*, 1993). Analysis of the function of the wheat gene in yeast depends first on tetrad analysis, since the FAS3 mutation is lethal in homozygotes.

Observation of four viable spores from FAS3 tetrads containing the wheat ACC gene may confirm that the wheat gene functions in yeast, and extracts of the complemented FAS3 mutant may be prepared and assayed for ACC activity. These assays may indicate the range of herbicide sensitivity, and in these studies, haloxyfop  
20 acid and clethodim may be used as well as other related herbicide compounds.

Given that the enzyme expressed in yeast is herbicide-sensitive, the present invention may be used in the isolation of herbicide-resistant mutants. If spontaneous mutation to resistance is too infrequent, chemical mutagenesis with DES or EMS may be used to increase such frequency. Protocols involving chemical mutagenesis are  
25 well-known to those of skill in the art. Resistant mutants, *i.e.*, strains capable of growth in the presence of herbicide, may be assayed for enzyme activity *in vitro* to verify that the mutation to resistance is within the ACC coding region.

Starting with one or more such verified mutants, several routes may lead to the identification of the mutated site that confers resistance. Using the available  
30 restriction map for the wild-type cDNA, chimeric molecules may be constructed



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containing half, quarter and eighth fragments, *etc.* from each mutant, then checked by transformation and tetrad analysis whether a particular chimera confers resistance or not.

Alternatively a series of fragments of the mutant DNA may be prepared, end-labeled, and annealed with the corresponding wild-type fragments in excess, so that all mutant fragments are in heterozygous molecules. Brief S1 or mung bean nuclease digestion cuts the heterozygous molecules at the position of the mismatched base pair. Electrophoresis and autoradiography is used to locate the position of the mismatch within a few tens of base pairs. Then oligo-primed sequencing of the mutant DNA is used to identify the mutation. Finally, the mutation may be inserted into the wild-type sequence by oligo-directed mutagenesis to confirm that it is sufficient to confer the resistant phenotype.

Having identified one or more mutations in this manner, the corresponding parts of several dicot ACC genes may be sequenced (using the physical maps and partial sequences as guides) to determine their structures in the corresponding region, in the expectation that they are now herbicide resistant.

#### 5.10 EXAMPLE 10 -- Isolation and Sequence Analysis of Canola ACC cDNA

Wheat ACC cDNA probes were used to detect DNA encoding canola ACC. Southern analysis indicated that a wheat probe hybridizes quite strongly and cleanly with only a few restriction fragments that were later used to screen canola cDNA and genomic libraries (both libraries provided by Pioneer HiBred Co [Johnson City, IA]). About a dozen positive clones were isolated from each library.

Sequence analysis was performed for several of these genomic clones. Fragments containing both introns and exons were identified. One exon sequence encodes a polypeptide which is 75% identical to a fragment of wheat ACC. This is very high conservation especially for this fragment of the ACC sequence which is not very conserved in other eukaryotes. The 398-nucleotide DNA segment comprising a portion of the canola ACC gene is given in SEQ ID NO:19. The 132-amino acid

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translated sequence comprising a portion of the canola ACC polypeptide is given in SEQ ID NO:20.

One of the other genomic clones (6.5 kb in size) contains the 5' half of the canola gene, and additional screening of the genomic library may produce other clones which contain the promoter and other potential regulatory elements.

#### 5.11 EXAMPLE 11 -- Methods for Obtaining ACC Mutants

In *E. coli*, only conditional mutations can be isolated in the *acc* genes. The reason is that although the bacteria can replace the fatty acids in triglycerides with exogenously provided ones, they also have an essential wall component called lipid A, whose  $\beta$ -hydroxy myristic acid can not be supplied externally.

One aspect of the present invention is the isolation of *Anacystis* mutants in which the BC gene is interrupted by an antibiotic resistance cassette. Such techniques are well-known to those of skill in the art (Golden *et al.*, 1987). Briefly, the method involves replacing the cyanobacterial ACC with wheat ACC, so it is not absolutely necessary to be able to maintain the mutants without ACC. The wheat ACC clone may be introduced first and then the endogenous gene can be inactivated without loss of viability.

By replacing the endogenous herbicide resistant ACC in cyanobacteria with the wheat cDNA, resulting cells are sensitive to the herbicides haloxyfop and clethodim, whose target is known to be ACC. Subsequently, one may isolate mutants resistant to those herbicides. These methods are known to those of skill in the art (Golden *et al.*, 1987).

The transformation system in *Anacystis* makes it possible to pinpoint a very small DNA fragment that is capable of conferring herbicide resistance. DNA sequencing of wild type and resistant mutants then reveals the basis of resistance.

Alternatively, gene replacement may be used to study wheat ACC activity and herbicide inhibition in yeast. Mutants may be selected which overcome the normal sensitivity to herbicides such as haloxyfop. This will yield a variant(s) of wheat ACC that are tolerant/resistant to the herbicides. The mutated gene (cDNA) present on the

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plasmid can be recovered and analyzed further to define the sites that confer herbicide resistance. As for the herbicide selection, there is a possibility that the herbicide may be inactivated before it can inhibit ACCase activity or that it may not be transported into yeast. There are general schemes for treatment of yeast with permeabilizing antibiotics at sublethal concentrations, which are known to those of skill in the art. Such treatments allow otherwise impermeable drugs to be used effectively. For these studies haloxyfop acid and clethodim may be used.

Characterization of the site(s) conferring herbicide resistance generally involves assaying extracts of the complemented ACC1 mutant for ACCase activity. Both spontaneous mutation and chemical mutagenesis with DES or EMS, may be used to obtain resistant mutants, *i.e.*, strains capable of growth in the presence of herbicide. These may be assayed for enzyme activity *in vitro* to verify that the mutation to resistance is within the ACCase coding region. Starting with one or more such verified mutants, the mutated site that confers resistance may be analyzed. Using the available restriction map for the wild-type cDNA, chimeric molecules may be constructed which containing half, quarter and eighth fragments, *etc.*, from each mutant, and then checked by transformation and tetrad analysis to determine whether a particular chimera confers resistance or not.

An alternative method involves preparing a series of fragments of the mutant DNA, end-labeling, and annealing with the corresponding wild-type fragments in excess, so that all mutant fragments are in heterozygous molecules. Brief S1 or mung bean nuclease digestion cuts the heterozygous molecules at the position of the mismatch within a few tens of base pairs. Then oligo-primed sequencing of the mutant DNA is used to identify the mutation. Finally, the mutation can be inserted into the wild-type sequence by oligo-directed mutagenesis to confirm that it is sufficient to confer the resistant phenotype. Having identified one or more mutations in this manner, the corresponding parts of several dicot ACCase genes to determine their structures in the corresponding region, in the expectation that they would be "resistant".

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Another method for the selection of wheat ACCase mutants tolerant or resistant to different herbicides involves the phage display technique. Briefly, in the phage display technique, foreign peptides can be expressed as fusions to a capsid protein of filamentous phage. Generally short (6 to 18 amino acids), variable amino acid sequences are displayed on the surface of a bacteriophage virion (a population of phage clones makes an epitope library). However, filamentous bacteriophages have also been used to construct libraries of larger proteins such as the human growth hormone, alkaline phosphatase (Scott, 1992) or a 50-kDa antibody Fab domain (Kang *et al.*, 1991). In those cases, the foreign inserts were spliced into the major coat protein pVIII of the M13 phagemid. A complementary helper phage supplying wild-type pVIII has to be cotransferred together with the phagemid. Such "fusion phages" retained full infectivity and the fused proteins were recognized by monoclonal antibodies. These results demonstrate that foreign domains displayed by phage can retain at least partial native folding and activity.

Phage libraries displaying wild-type fragments of the wheat ACCase of 250 to 300 amino acids in size may be constructed without "panning" for phage purification. The mechanism of purifying phages by panning involves reaction with biotinylated monoclonal antibodies, then the complexes are diluted, immobilized on streptavidin-coated plates, washed extensively and eluted. Generally, a few rounds of panning are recommended.

Instead, fragments bearing the ATP-binding site may be obtained by using Blue Sepharose CL-6B affinity chromatography, which was shown to bind plant ACCs (Betty *et al.*, 1992; Egin-Buhler *et al.*, 1980). Herbicides bound to Sepharose serve for capturing those phages which display amino acid fragments involved in herbicide binding. Such herbicide affinity resins may also be employed. After identifying peptide fragments that bind herbicides, ATP or acetyl-CoA, the phages bearing those peptides may be subjected to random mutagenesis, again using phage display and binding to the appropriate support to select the interesting variants. Sequence analysis then is used to identify the critical residues of the protein required for binding.

### 5.12 EXAMPLE 12 -- Preparation of ACC-specific antibodies

Another aspect of the present invention is the preparation of antibodies reactive against plant ACC for use in immunoprecipitation, affinity chromatography, and immunoelectron microscopy. The antisera may be prepared in rabbits, using methods that are well-known to those of skill in the art (see *e.g.*, Schneider and Haselkorn, 1988).

Briefly, the procedure encompasses the following aspects. Gel-purified protein is electroeluted, dialyzed, mixed with complete Freund's adjuvant and injected in the footpad at several locations. Subsequent boosters are given with incomplete adjuvant and finally with protein alone. Antibodies are partially purified by precipitating lipoproteins from the serum with 0.25% sodium dextran sulfate and 80 mM CaCl<sub>2</sub>. Immunoglobulins are precipitated with 50% saturating ammonium sulfate, suspended in phosphate-buffered saline at 50 mg/ml and stored frozen. The antisera prepared as described may be used in Western blots of protein extracts from wheat, pea, soybean, canola and sunflower chloroplasts as well as total protein.

### 5.13 EXAMPLE 13 -- Protein Fusions, Transgenic Plants and Transport Mutants

Analysis of promoter and control elements with respect to their structure as well as tissue specific expression, timing *etc.*, is performed using promoter fusions (*e.g.* with the GUS gene) and appropriate *in situ* assays. Constructs may be made which are useful in the preparation of transgenic plants.

For identifying transport of ACC, model substrates containing different length N-terminal fragments of ACC may be prepared by their expression (and labeling) in *E. coli* or by *in vitro* transcription with T7 RNA polymerase and translation (and labeling) in a reticulocyte lysate. Some of the model substrates will include the functional biotinylation site (located ~800 amino acids from the N-terminus of the mature protein; the minimum biotinylation substrate will be defined in parallel) or native ACC epitope(s) for which antibodies will be generated as described above.

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Adding an antibody tag at the C-terminus will also be very helpful. These substrates will be purified by affinity chromatography (with antibodies or streptavidin) and used for *in vitro* assays.

For modification of ACC protein transport, model substrates consisting of a transit peptide (or any other chloroplast targeting signals) to facilitate import into chloroplasts, fused to different ACC domains that are potential targets for modification, may be used. Modified polypeptides from cytoplasmic and/or chloroplast fractions will be analyzed for modification. For example, protein phosphorylation (with  $^{32}\text{P}$ ) can be followed by immunoprecipitation or by PAGE. Antibodies to individual domains of ACC may then be employed. The same experimental set-up may be employed to study the possible regulation of plant ACC by phosphorylation (*e.g.*, Witters and Kemp, 1992). Biotinylation may be followed by Western analysis using  $^{35}\text{S}$ -streptavidin for detection or by PAGE when radioactive biotin is used as a substrate.

#### 5.14 EXAMPLE 14 -- Expression Systems for Preparation of ACC Polypeptides

The entire plant ACC cDNA and its fragments, and BC, BCCP and the CT gene clones from cyanobacteria may be used to prepare large amounts of the corresponding proteins in *E. coli*. This is most readily accomplished using the T7 expression system. As designed by Studier, this expression system consists of an *E. coli* strain carrying the gene for T7 lysozyme and for T7 RNA polymerase, the latter controlled by a *lac* inducible promoter. The expression vector with which this strain can be transformed contains a promoter recognized by T7 RNA polymerase, followed by a multiple cloning site into which the desired gene can be inserted (Ashton *et al.*, 1994).

Prior to induction, the strain grows well, because the few molecules of RNA polymerase made by basal transcription from the *lac* promoter are complexed with T7 lysozyme. When the inducer IPTG is added, the polymerase is made in excess and the plasmid-borne gene of interest is transcribed abundantly from the late T7 promoter.

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This system easily makes 20% of the cell protein the product of the desired gene. A benefit of this system is that the desired protein is often sequestered in inclusion bodies that are impossible to dissolve after the cells are lysed. This is an advantage in the present invention, because biological activity of these polypeptides is not required for purposes of raising antisera. Moreover, other expression systems are also available (Ausubel *et al.*, 1989).

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## 7. SEQUENCE LISTING

## (1) GENERAL INFORMATION:

5

## (i) APPLICANT:

- (A) NAME: ARCH DEVELOPMENT CORPORATION
- (B) STREET: 1101 East 58th Street
- (C) CITY: Chicago
- 10 (D) STATE: Illinois
- (E) COUNTRY: United States of America
- (F) POSTAL CODE (ZIP): 60637

15

(ii) TITLE OF INVENTION: NUCLEIC ACID COMPOSITIONS  
ENCODING ACETYL-CoA  
CARBOXYLASE AND USES  
THEREFOR

20

(iii) NUMBER OF SEQUENCES: 40

## (iv) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- 25 (D) SOFTWARE: PatentIn Release #1.0, Version  
#1.30 (EPO)

30

## (vi) PRIOR APPLICATION DATA:

- (A) APPLICATION NUMBER: US Unknown
- (B) FILING DATE: 05-MAR-1996

35

## (vi) PRIOR APPLICATION DATA:

- (A) APPLICATION NUMBER: US 08/422,560
- (B) FILING DATE: 14-APR-1995

## (2) INFORMATION FOR SEQ ID NO: 1:

-114-

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1458 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

AAGCTTCATG ATTTCTAGTA ACGATTTTCG ACCTGGTGTA TCCATTGTCT TAGATGGGTC 60

10

TGTATGGCGA GTGATAGATT TCCTTCACGT TAAGCCAGGT AAGGGTTCTG CCTTGTACG 120

GACAACTCTG AAGAACGTCC AAAGCGGCAA AGTTTATAGAA AAAACCTTCC GGGCTGGGGA 180

15 AACTGTTCCA CAAGCTACTT TAGAAAAAAT TACAATGCAG CATACTATA AAGAGGCGGA 240

TGAGTTCGTC TTTATGGATA TGGAAAGCTA TGAAGAAGGA CGACTCAGCG CCGCACAAAT 300

TGGCGATCGC GTCAAATACC TCAAGGAAGG TATGGAAGTG AACGTCATTC GTTGGGGTGA 360

20

GCAAGTGCTA GAGGTGGAAC TGGCTAATTC TGTAGTCTTG GAAGTTATAC AAACGTGATCC 420

AGGTGTCAAG GGTGACACGG CTACAGGTGG CACGAAACCA GCAATTGTG AACTGGTGC 480



-115-

AACTGTGATG GTTCCTTTGT TTATTTCTCA AGGAGAGCGA ATTAAAATTG ATACCCGTGA 540  
TGATAAATAC TTAGGCAGGG AATAGGTTTT ATCTCATCCG AGAACAAATC CCGATTTCAA 600  
5 TCCCTATTTC AGGGATTAAA TCCCTGCCAC ACTTAGGCCA ATTCAAAATT CAAAATTCAA 660  
AAACTGGAT TCCCTTAAGG TTTCTGAGTC TCAATGGTAG ATGGATTTTG GAGAGTTGGT 720  
ATGAAAAAAT CTTTATTTAC GGA CTGGTCG AGGTAATAAA AACTGTGCCA TTGGACTTTA 780  
10 ATGAAATCCG TCAACTGCTG ACAA CTATTG CACAAACAGA TATCGCGGAA GTAACGCTCA 840  
AAAGTGATGA TTTTGAACTA ACGGTGCCGA AAGCTGTTGG TGTGAATAAT AGTGTGTGTC 900  
15 CGGTTGTGAC AGCACCCCTTG AGTGGTGTGG TAGGTTCCGG ATTGCCATCG GCTATACCGA 960  
TTGTAGCCCA TGCTGCCCCA TCTCCATCTC CAGAGCCGGG AACAAAGCCGT GCTGCTGATC 1020  
ATGCTGTAC GAGTTCTGGC TCACAGCCAG GAGCAAAAAT CATTGACCAA AAATTAGCAG 1080  
AAGTGGCTTC CCCAATGGTG GGAACATTTT ACCGGGCTCC TGCACCAGGT GAAGCGGTAT 1140  
20 TTGTGGAAGT CGGGGATCGC ATCCGTCAAG GTCAAAACCGT CTGCATCATC GAAGCAATGA 1200

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AGCTGATGAA TGAAATTGAG GCTGATGTTT CTGGGCAAGT GATCGAAATT CTCGTCCAAA 1260  
 ACGGCGAACC TGTAATAAT AATCAACCTT TGATGAGAAT TAAACCAGAT TAAGTATTAA 1320  
 5 TGTATATAGG TGAGTCATTA CTAACCTCAAG TTGCTAGTTA TGTTTGGTAA TTGGTAACTG 1380  
 GTGATTGCTA ATTGGTAAT GAGAAAAAATT TTAATCATTG CCCATCACCC ATTACCAGTT 1440  
 CTTAAATTGA TAGCTAGC 1458

10

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 182 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

20

Met Pro Leu Asp Phe Asn Glu Ile Arg Gln Leu Leu Thr Thr Ile Ala  
 1 5 10 15

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Gln Thr Asp Ile Ala Glu Val Thr Leu Lys Ser Asp Phe Glu Leu  
20 25 30

Thr Val Arg Lys Ala Val Gly Val Asn Asn Ser Val Val Pro Val Val  
35 40 45

Thr Ala Pro Leu Ser Gly Val Val Gly Ser Gly Leu Pro Ser Ala Ile  
50 55 60

Pro Ile Val Ala His Ala Ala Pro Ser Pro Ser Pro Glu Pro Gly Thr  
65 70 75 80

Ser Arg Ala Ala Asp His Ala Val Thr Ser Ser Gly Ser Gln Pro Gly  
85 90 95

Ala Lys Ile Ile Asp Gln Lys Leu Ala Glu Val Ala Ser Pro Met Val  
100 105 110

Gly Thr Phe Tyr Arg Ala Pro Ala Pro Gly Glu Ala Val Phe Val Glu  
115 120 125

Val Gly Asp Arg Ile Arg Gln Gly Gln Thr Val Cys Ile Ile Glu Ala  
130 135 140

-118-

Met Lys Leu Met Asn Glu Ile Glu Ala Asp Val Ser Gly Gln Val Ile  
 145 150 155 160

Glu Ile Leu Val Gln Asn Gly Glu Pro Val Glu Tyr Asn Gln Pro Leu  
 165 170 175

Met Arg Ile Lys Pro Asp  
 180

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 477 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

GTGCAACTGA ACTTCAGCCA ACTGCAAGAG CTGCTGACCG TGCTGAGTGA CTCAGACATC 60

GCTGAGTTTG ACCTCAAAGG TACGGATTTT GAGTTGCACG TGAAGCGCGG CTCGACCGGC 120

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GACCCGATCG TCATTGCGGC TCCCACCACG CCCGTTGCTG TCGTCCCCTG GCCCGCTCCC 180  
TTACCCGCTC CAACCCCTGC GGCAGACCG CCTGCTGGAC CTCGTGGTGG CGAGAAGTTC 240  
5 CTTGAGATTA CGGCGCCGAT GGTGGGCACC TTCTATCGCG CTCAGACACC GGAAGAACCG 300  
CCCTTCGTCA ATGTTGGCA TCGATTTCAG GTGGGACAGA CCGTCTGCAT CCTCGAAGCG 360  
ATGAAGCTGA TGAACGAGTT GGAGTCGGAG GTGACGGGGG AAGTCGTCGA GATTCTGGTC 420  
10 CAGAACGGCG AACCGGTGA GTTAATCAG CCCCTGTTCC GGTGCGGCC TCTCTGA 477

15 (2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 158 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

-120-

Met Gln Leu Asn Phe Ser Gln Leu Gln Leu Thr Val Leu Ser  
1 5 10 15

Asp Ser Asp Ile Ala Glu Phe Asp Leu Lys Gly Thr Asp Phe Glu Leu  
5 20 25 30

His Val Lys Arg Gly Ser Thr Gly Asp Pro Ile Val Ile Ala Ala Pro  
35 40 45

Thr Thr Pro Val Ala Val Ala Pro Val Pro Ala Pro Leu Pro Ala Pro  
10 50 55 60

Thr Pro Ala Ala Pro Pro Ala Gly Pro Leu Gly Gly Glu Lys Phe  
65 70 75 80

Leu Glu Ile Thr Ala Pro Met Val Gly Thr Phe Tyr Arg Ala Pro Ala  
85 90 95

Pro Glu Glu Pro Pro Phe Val Asn Val Gly Asp Arg Ile Gln Val Gly  
100 105 110

Gln Thr Val Cys Ile Leu Glu Ala Met Lys Leu Met Asn Glu Leu Glu  
115 120 125

-121-

Ser Glu Val Thr Gly Glu Val Val Glu Ile Leu Val Gln Asn Gly Glu  
130 135 140

Pro Val Glu Phe Asn Gln Pro Leu Phe Arg Leu Arg Pro Leu  
145 150 155

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

- 10 (A) LENGTH: 3065 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

AAGCTTTTAT ATTTGCCAT TTCTAGAACT TAGCTGCATC GGCCCCAAGT ATTTTGTCAA 60  
ATATGGCGAA AAGACTTCAT AAATCAAGGT TAAAGGTTGA CCGTGATGCC AAAACAGGTA 120  
ATGGCGACCC CAGAAAGGCC CATCCACGCC AAAACCTAAT TGCAAGGCCT CTGAATTTC 180  
GTAATAAATA CCGGCACAT CCGGATACAA CTCGCTGCGA AGACGAGCTA GACTTGCCCA 240

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-122-

300 AATTGGTAAT GAACGGTTTT GCAAATACTC GTCTACATGG CTGGCTTCCC ACCATGAGGT  
360 TGCATAGGCG AGTCGTTGGC CAGAGCGTGT ACGTAGCCAT ACCTGTCGCC GCAGTCTTGG  
420 CGCTGGAACA GATTGGATTA AATCCGGGCG ACTATCTAAA TCCAAACCAA TCAATGACAT  
480 ATCAATGACA TCGACTTCTG TTGGCTCACC AGTAAGTAAT TCTAAATGCC TTGTGGGTGA  
540 GCCATCACCT AAGAGTAGTA GTTGCCACGC TGGAGCCAGC TGAGTGTGAG GCAAACTATG  
600 TTTAATTACT TCTTCCCAC CTTGCCAAAT AGGAGTGAGG CGATGCCAATC CGGCTGGCAG  
660 TGTGAGTTG TTGCTTGGAG TAAAAGTGGC AGTCAATGTT CTTTACAAAA GTTCACCTAT  
720 TTATATCAAA GCATAAAAA TTAATTAGTT GTCAGTTGTC ATTGGTTATT CTTCTTTGCT  
780 CCCCCTGCCC CCTACTTCCC TCCTCTGCCC AATAATTAGA AAGGTCAGGA GTCAAAAACT  
840 TATCACTTTT GACCACTGAC CTTTCACAAT TGACTATAGT CACTAAAAAA TGCGGATGGC  
900 GAGACTCGAA CTGCGAAGGC AAAGCCACAC GCACCTCAAG CGTGCGCGTA TACCAATTCC  
960 GCCACATCCG CACGGGTTGT ACAAGAAGAT ATACTAGCAC AAAAAAATTG CATAAAACAA

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-123-

GGTAAACTA TATTTGCCAA ACTTTATGGA AAATTTATCT TGCTAAATAT ACAAATTTCC 1020  
CGAAGAGGAT ACGAGACTAA CAGAAATGTA GTATCGCCAC AAGTGATATT AAAGGGGGTA 1080  
5 TGGGGGTTTT CTTCCCTTAC ACCCTTAAAC CCTCACACCC CACCTCCATG AAAAATCTTG 1140  
TTGTAAGTC CGTTTCCTGC AATTTATTTA AAGATGAGCC TGGGGTATCT CCTGTCATAA 1200  
TTTGAGATGA AGCGATGCCT AAGGCGGCTA CGCTACGCGC TAAAAGCAAC TTGGATGGGA 1260  
GACAATTTCT ATCTGCTGGT ACTGATACTG ATATCGAAAA CTAGAAAAATG AAGTTTGACA 1320  
AAATATTAAT TGCCAATCGG GGAGAAATAG CGCTGCGCAT TCTCCGCGCC TGTGAGGAAA 1380  
15 TGGGGATTGC GACGATCGCA GTTCATTCCA CTGTTGACCG GAATGCTCTT CATGTCCAAC 1440  
TTGCTGACGA AGCGGTTTGT ATTGGCGAAC CTGCTAGCGC TAAAAGTTAT TTGAATATTTC 1500  
CCAATATTAT TGCTGCGGCT TTAACGCGCA ATGCCAGTGC TATTCATCCT GGGTATGGCT 1560  
TTTTATCTGA AAATGCCAAA TTTGGGGAAA TCTGTGCTGA CCATCACATT GCATTCAATTG 1620  
GCCCCACCCC AGAAGCTATC CGCCTCATGG GGGACAAATC CACTGCCAAG GAAACCATGC 1680  
20

-124-

AAAAAGCTGG TGTACCGACA GTACCGGGTA GTGAAGGTTT GGTAGAGACA GAGCAAGAAG 1740  
GATTAGAACT GCGGAAAGAT ATTGGCTACC CAGTGATGAT CAAAGCCACG GCTGGTGGTG 1800  
GCGGCGGGG TATGCGACTG GTGCGATCGC CAGATGAATT TGTCAAAC TGTTAGCCG 1860  
CCCAAGGTGA AGCTGGTGCA GCCTTTGGTA ATGCTGGCGT TTATATAGAA AAATTTATTG 1920  
AAGTCCCGG CCACATTGAA TTTCAAATTT TGGCTGATAA TTACGGCAAT GTGATTCACT 1980  
TGGGTGAGAG GGATTGCTCA ATTACGCGTC GTAACCAAAA GTTACTAGAA GAAGCCCCCA 2040  
GCCCAGCCTT GGA CTCAGAC CTAAGGGAAA AAATGGGACA AGCGCGGGTG AAAGCGGCTC 2100  
AGTTTATCAA TTACGCGGGG GCAGGTACTA TCGAGTTTTT GCTAGATAGA TCCGGTCAGT 2160  
TTTACTTTAT GGAGATGAAC ACCCGGATTC AAGTAGAACA TCCCGTAACT GAGATGGTTA 2220  
CTGGAGTGGA TTTATTGGTT GAGCAAAATCA GAATTGCCCA AGGGGAAAGA CTTAGACTAA 2280  
CTCAAGACCA AGTAGTTTTA CGCGGTCATG CGATCGAATG TCGCATCAAT GCCGAAGACC 2340  
CAGACCACGA TTTCCGCCCA GCACCCGGAC GCATTAGCGG TTATCTTCCC CCTGGCGGCC 2400

10

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-125-

CTGGCGTGG GATTGACTCC CAGTTTACA CGGATTACCA AATCCGCCCC TACTACGATT 2460  
CCTTAATTGG TAAATTGATC GTTTGGGGCC CTGATCGCGC TACTGCTATT AACCGCATGA 2520  
5 AACGCGCCCT CAGGGAATGC GCCATCACTG GATTACCTAC AACCATTGGG TTTCATCAAA 2580  
GAATTATGGA AAATCCCCAA TTTTACAAG GTAATGTGTC TACTAGTTTT GTGCAGGAGA 2640  
TGAATAAATA GGGTAATGGG TAATGGGTAA TGGTAATAG AGTTTCAATC ACCAATTACC 2700  
AATTCCTTAA CTCATCCGTG CCAACATCGT CAGTAATCCT TGCTGGCCTA GAAGAACTTC 2760  
TCGCAACAGG CTAAAAATAC CAACACACAC AATGGGGTG ATATCAACAC CACCTATTGG 2820  
TGGGATGATT TTTCGCAAGG GAATGAGAAA TGGTTCAGTC GGCCAAGCAA TTAAGTTGAA 2880  
GGGCAACAGG TTCAGATCGA CTTCGGGATA CCAGGTCAGA ATGATACGGA AAATAAACAG 2940  
AAATGTATC ACTCCCAATA CAGGGCCAAG AATCCAAAG CTCAGGTAA CACCAGTCAT 3000  
20 CGATCTAAGC TACTATTTTG TGAATTTACA AAAAATGCA AGCAAAAGCT GAAAATTTTA 3060  
AGCTT 3065

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## (2) INFORMATION FOR SEQ ID NO: 6:

## (i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 447 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS:  
 (D) TOPOLOGY: linear

## 10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Met Lys Phe Asp Lys Ile Leu Ile Ala Asn Arg Gly Glu Ile Ala Leu  
 1 5 10 15  
 Arg Ile Leu Arg Ala Cys Glu Glu Met Gly Ile Ala Thr Ile Ala Val  
 20 25 30  
 His Ser Thr Val Asp Arg Asn Ala Leu His Val Gln Leu Ala Asp Glu  
 35 40 45  
 Ala Val Cys Ile Gly Glu Pro Ala Ser Ala Lys Ser Tyr Leu Asn Ile  
 50 55 60

20

-127-

Pro Asn Ile Ile Ala Ala Ala Leu Thr Arg Asn Ala Ser Ala Ile His  
 65 70 75 80

Pro Gly Tyr Gly Phe Leu Ser Glu Asn Ala Lys Phe Ala Glu Ile Cys  
 85 90 95

Ala Asp His His Ile Ala Phe Ile Gly Pro Thr Pro Glu Ala Ile Arg  
 100 105 110

Leu Met Gly Asp Lys Ser Thr Ala Lys Glu Thr Met Gln Lys Ala Gly  
 115 120 125

Val Pro Thr Val Pro Gly Ser Glu Gly Leu Val Glu Thr Glu Gln Glu  
 130 135 140

Gly Leu Glu Leu Ala Lys Asp Ile Gly Tyr Pro Val Met Ile Lys Ala  
 145 150 155 160

Thr Ala Gly Gly Gly Arg Gly Met Arg Leu Val Arg Ser Pro Asp  
 165 170 175

Glu Phe Val Lys Leu Phe Leu Ala Ala Gln Gly Glu Ala Gly Ala Ala  
 180 185 190

5

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-128-

Phe Gly Asn Ala Gly Val Tyr Ile Glu Lys Phe Ile Glu Arg Pro Arg  
 195 200 205

His Ile Glu Phe Gln Ile Leu Ala Asp Asn Tyr Gly Asn Val Ile His  
 210 215 220

Leu Gly Glu Arg Asp Cys Ser Ile Gln Arg Arg Asn Gln Lys Leu Leu  
 225 230 235 240

10 Glu Glu Ala Pro Ser Pro Ala Leu Asp Ser Asp Leu Arg Glu Lys Met  
 245 250 255

Gly Gln Ala Ala Val Lys Ala Ala Gln Phe Ile Asn Tyr Thr Gly Ala  
 260 265 270

15 Gly Thr Ile Glu Phe Leu Leu Asp Arg Ser Gly Gln Phe Tyr Phe Met  
 275 280 285

Glu Met Asn Thr Arg Ile Gln Val Glu His Pro Val Thr Glu Met Val  
 290 295 300

Thr Gly Val Asp Leu Leu Val Glu Gln Ile Arg Ile Ala Gln Gly Glu  
 305 310 315 320

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Arg Leu Arg Leu Thr Gln Asp Gln Val Val Leu Arg Gly His Ala Ile  
325 330 335

Glu Cys Arg Ile Asn Ala Glu Asp Pro Asp His Asp Phe Arg Pro Ala  
340 345 350

Pro Gly Arg Ile Ser Gly Tyr Leu Pro Pro Gly Gly Pro Gly Val Arg  
355 360 365

10 Ile Asp Ser His Val Tyr Thr Asp Tyr Gln Ile Pro Pro Tyr Tyr Asp  
370 375 380

Ser Leu Ile Gly Lys Leu Ile Val Trp Gly Pro Asp Arg Ala Thr Ala  
385 390 395 400

Ile Asn Arg Met Lys Arg Ala Leu Arg Glu Cys Ala Ile Thr Gly Leu  
405 410 415

20 Pro Thr Thr Ile Gly Phe His Gln Arg Ile Met Glu Asn Pro Gln Phe  
420 425 430

Leu Gln Gly Asn Val Ser Thr Ser Phe Val Gln Glu Met Asn Lys  
435 440 445

-130-

## (2) INFORMATION FOR SEQ ID NO: 7:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1362 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

5  
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ATGCGTTTCA ACAAGATCCT GATCGCCAAT CGCGGCGAAA TCGCCCTGCG CATTCCTCGC 60  
ACTTGTGAAG AACTCGGGAT CGGCACGATC GCCGTTCACT CCACTGTGGA TCGCAACGCG 120  
CTCCATGTGC AGTTAGCGGA CGAAGCGGTC TGTATTGGCG AAGCGGCCAG CAGCAAAAGC 180  
TATCTCAATA TCCCCAACAT CATTGCGGCG GCCCTGACCC GTAATGCCAG CGCCATTAC 240  
CCCCGCTATG GCTTCTTGGC GGAGAATGCC CGCTTTGCAG AAATCTGCGC CGATCACCAT 300  
CTCACCTTTA TTGGCCCCCAG CCCCATTTCG ATTCGAGCCA TGGGCGATAA ATCCACCGCT 360  
AAGGAACAA TGCAGCGGT CGGCGTTCCG ACGATTCCGG GCAGTGACGG TCTGCTGACG 420



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480 GATGTTGATT CGGCTGCCAA AGTTGCTGCC GAGATCGGCT ATCCCGTCAT GATCAAGCG  
540 ACGGCGGGG GCGGTGGTCG CCGTATGCGG CTGGTGGTG AGCCTGCAGA TCTGAAAAA  
600 CTGTTCCCTTG CTGCCCAAGG AGAAGCCGAG GCAGCTTTTG GGAATCCAGG ACTGTATCTC  
660 GAAAAATTTA TCGATCGCCC ACGCCACGTT GAATTTTCTG TCTTGCCGA TGCCTACGGC  
720 AATGTAGTGC ATCTAGGCGA GCGCGATTGC TCCATTCAAC GTCGTCACCA AAAGCTGCTC  
780 GAAGAAGCCC CCAGTCCGGC GCTATCGGCA GACCTGCGGC AGAAAATGG CGATGCCGCC  
840 GTCAAAGTCG CTCAAGCGAT CCGCTACATC GGTGCCGGCA CCGTGGAGTT TCTGGTCGAT  
900 GCGACCGGCA ACTTCTACTT CATGGAGATG AATACCCGCA TCCAAGTCGA GCATCCAGTC  
960 ACAGAAATGA TTACGGGACT GGACTTGATT GCGGAGCAGA TTCGGATTGC CCAAGGCGAA  
1020 GCGCTGCGCT TCCGGCAAGC CGATATTCAA CTGCGCGGCC ATGCGATCGA ATGCCGTATC  
1080 AATGCGGAAG ATCCGGAATA CAATTTCGG CCGAATCCTG GCCGCATTAC AGGCTATTTA  
1140 CCGCCCGCGG GCCCGGCGT TCGTGTGAT TCCCATGTTT ATACCGACTA CGAAATTCCG

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-132-

CCCTATTACG ATTCGCTGAT TGGCAATATG ATTGTCTGGG GTGCAACACG GGAAGAGGCG 1200  
ATCGCGGGGA TGCAGCGTGC TCTGCGGGAA TGGGCCATCA CCGGCTTGCC GACGACCCTT 1260  
5 AGTTTCCATC AGCTGATGTT GCAGATGCCT GAGTTCCTGC GCGGGGAACT CTATACCAAC 1320  
TTTGTGAGC AGGTGATGCT ACCTCGGATC CTCAGTCTCT AG 1362

10 (2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 453 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS:  
(D) TOPOLOGY: linear

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

20 Met Arg Phe Asn Lys Ile Leu Ile Ala Asn Arg Gly Glu Ile Ala Leu  
1 5 10 15

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Arg Ile Leu Arg Thr Cys Glu Glu Leu Gly Ile Gly Thr Ile Ala Val  
20 25 30

His Ser Thr Val Asp Arg Asn Ala Leu His Val Gln Leu Ala Asp Glu  
35 40 45

Ala Val Cys Ile Gly Glu Ala Ala Ser Ser Lys Ser Tyr Leu Asn Ile  
50 55 60

Pro Asn Ile Ile Ala Ala Leu Thr Arg Asn Ala Ser Ala Ile His  
65 70 75 80

Pro Gly Tyr Gly Phe Leu Ala Glu Asn Ala Arg Phe Ala Glu Ile Cys  
85 90 95

Ala Asp His His Leu Thr Phe Ile Gly Pro Ser Pro Asp Ser Ile Arg  
100 105 110

Ala Met Gly Asp Lys Ser Thr Ala Lys Glu Thr Met Gln Arg Val Gly  
115 120 125

Val Pro Thr Ile Pro Gly Ser Asp Gly Leu Leu Thr Asp Val Asp Ser  
130 135 140

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Ala Ala Lys Val Ala Ala Glu Ile Gly Tyr Pro Val Met Ile Lys Ala  
145 150 155 160

Thr Ala Gly Gly Gly Arg Gly Met Arg Leu Val Arg Glu Pro Ala  
165 170 175

Asp Leu Glu Lys Leu Phe Leu Ala Ala Gln Gly Glu Ala Glu Ala Ala  
180 185 190

10 Phe Gly Asn Pro Gly Leu Tyr Leu Glu Lys Phe Ile Asp Arg Pro Arg  
195 200 205

His Val Glu Phe Gln Ile Leu Ala Asp Ala Tyr Gly Asn Val Val His  
210 215 220

15 Leu Gly Glu Arg Asp Cys Ser Ile Gln Arg Arg His Gln Lys Leu Leu  
225 230 235 240

Glu Glu Ala Pro Ser Pro Ala Leu Ser Ala Asp Leu Arg Gln Lys Met  
245 250 255

Gly Asp Ala Ala Val Lys Val Ala Gln Ala Ile Gly Tyr Ile Gly Ala  
260 265 270

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Gly Thr Val Glu Phe Leu Val Asp Ala Thr Gly Asn Phe Tyr Phe Met  
275 280 285

Glu Met Asn Thr Arg Ile Gln Val Glu His Pro Val Thr Glu Met Ile  
290 295 300

Thr Gly Leu Asp Leu Ile Ala Glu Gln Ile Arg Ile Ala Gln Gly Glu  
305 310 315 320

Ala Leu Arg Phe Arg Gln Ala Asp Ile Gln Leu Arg Gly His Ala Ile  
325 330 335

Glu Cys Arg Ile Asn Ala Glu Asp Pro Glu Tyr Asn Phe Arg Pro Asn  
340 345 350

Pro Gly Arg Ile Thr Gly Tyr Leu Pro Pro Gly Gly Pro Gly Val Arg  
355 360 365

Val Asp Ser His Val Tyr Thr Asp Tyr Glu Ile Pro Pro Tyr Tyr Asp  
370 375 380

Ser Leu Ile Gly Lys Leu Ile Val Trip Gly Ala Thr Arg Glu Glu Ala  
385 390 395 400

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Ile Ala Arg Met Gln Arg Ala Leu Arg Glu Cys Ala Ile Thr Gly Leu  
405 410 415

Pro Thr Thr Leu Ser Phe His Gln Leu Met Leu Gln Met Pro Glu Phe  
420 425 430

Leu Arg Gly Glu Leu Tyr Thr Asn Phe Val Glu Gln Val Met Leu Pro  
435 440 445

10 Arg Ile Leu Lys Ser  
450

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 7360 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

-137-

ATCTCTTTCA ACTTGGATAC CAGGCCGTTG CCTCCGCCGC CGCCGCCTGC CTGCCTCTCC 60  
TGGATCTCCA TCTCTCCTTC GCGGCGCGGC ATTCCGTGCA ACGCCTCCGC GCGCGGCCTC 120  
CGGGCGGACT CACGTGCTGA AGTTTGGAGG GGGCAATAAT GGTGGAATCT GACCAGATAA 180  
ACGGGAGGAT GTCTCTGGTC GACGAGTTCT GTAAAGCGCT CGGGGGCGAC TCGCCGATAC 240  
ACAGCGTGCT GGTGCGCAAC AATGGGATGG CTGCGGTCAA GTTCATGCGC AGCATCCGCA 300  
CCTGGGCCTT GGAGACCITT GGAACGAGA AGGCCATTCT CTGTTGGCT ATGGCAACTC 360  
CAGAGACCT CAGGATTAAT GCGGAGCACA TAAGAATCGC CGACCAGTTC TTAGAAGTTC 420  
CTGGTGGGAC GAATAACAAC AACTATGCAA ATGTACAGCT CATAGTGGAG ATAGCAGAGA 480  
GAACTCGGGT TTCTGCAGTT TGGCCTGGCT GGGTCAATGC TTCTGAGAAC CCAGAACTTC 540  
CAGACGGCT CATGGAAAAG GGAATCATT TTCTTGGGCC ACCATCAGCC GCGATGGGG 600  
CACTAGGCGA TAAGATTGGT TCTTCTCTTA TTGCACAAGC AGCAGGAGTT CCAACTCTTC 660  
CATGGAGCGG GTCACATGTG AAAGTTCCGC AAGAAACCTG CCACTCAATA CCTGAGGAGA 720

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780 TCTATAAGAA CGCTTGTGTT TCAACTACAG ACGAAGCAGT CGCTAGTTGT CAGGTGGTGG  
840 GGTATCCTGC AATGATCAAG GCATCATGGG GTGGGGGTGG TAAAGGAATA AGGAAGGTAC  
900 5 ACAATGATGA TGAGGTCAGA GCATTGTTTA AGCAAGTGCA AGGAGAGGTC CCCGGATCGC  
960 CTATATTAT TATGAAGGTG GCATCTCAGA GCCGACATCT AGAGGTTTCAG TTGCTCTGTG  
1020 ACAAGCATGG CAACGTGGCA GCACTGCACA GTCGAGACTG TAGTGTTCAA AGAAGGCACC  
1080 AAAAGATCAT TGAGGAGGGA CCAATTACAG TTGCTCCTCC AGAAACAATT AAAGAGCTTG  
1140 AGCAGGCGGC AAGGCGACTA GCTAAATGIG TGCAATATCA GGGTGCTGCT ACAGTGAAT  
1200 15 ATCTGTACAG CATGGAAACA GGCGAATACT ATTTCCTGGA GCTTAATCCA AGGTTGCAGG  
1260 TAGAACACCC TGTGACCGAA TGGATTGCTG AAATTAACTT ACCTGCATCT CAAGTTGTAG  
1320 TAGGAATGGG CATACCACTC TACAATATTC CAGAGATCAG ACGCTTTTAT GGAATAGAAC  
1380 ATGGAGGTGG CTATCAGCT TGAAGGAAA TATCAGCTGT AGCAACTAAA TTTGATTGG  
1440 20 ACAAAGCACA GTCTGTAAAG CCAAAGGGTC ATTGTGTAGC AGTTAGAGTT ACTAGCGAGG



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ATCCAGATGA TGGGTTTAAAG CCTACCAGTG GAAGAGTGGA AGAGCTGAAC TTAAAAAGCA 1500  
AACCCAATGT TTGGGCCTAC TTCTCCGTTA AGTCCGGAGG TGCAATTCAT GAGTTCTCTG 1560  
5 ATTCCCAGTT TGGTCATGTT TTTGCTTTTG GGAATCTAG GTCATTGGCA ATAGCCAATA 1620  
TGGTACTTGG GTTAAAAGAG ATCCAAATTC GTGGAGAGAT ACGCACTAAT GTTGACTACA 1680  
CTGTGGATCT CTTGAATGCT GCAGAGTACC GAGAAAATAA GATTCACACT GGTGGGCTAG 1740  
ACAGCAGAAAT AGCTATGCGT GTTAGAGCAG AGAGGCCCCC ATGGTACCCTT TCAGTTGTTG 1800  
GTGGAGCTCT ATATGAAGCA TCAAGCAGGA GCTCGAGCGT TGTAACCGAT TATGTTGGTT 1860  
15 ATCTCAGTAA AGGTCAAATA CCACCAAAGC ACATCTCTCT TGTCAATTG ACTGTGACAC 1920  
TGAATATAGA TGGGGGCAAA TATACGATTG AGACAGTACG AGGTGGACCC CGTAGCTACA 1980  
AATTAAGAAT TAATGAATCA GAGGTTGAAG CAGAGATACA TTCTCTGCCA GATGGCGGAC 2040  
TCITTAATGCA GTTGGATGGA AACAGTCAATG TAATTACGC CGAGACAGAA GCTGCTGGCA 2100  
CGCGCCTTCT AATCAATGGG AGAACATGCT TATTACAGAA AGAGCATGAT CCTTCCAGGT 2160  
20

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-140-

2220 TGTGGCTGA TACACCGTGC AAACCTCTTC GGTTTTGGT CGCGGATGGT TCTCATGTGG  
2280 TTGCTGATAC GCCATATGCT GAGGTGGAGG TTAAGAAAAT GTGCATGCCA CTGTTACTAC  
2340 CGGCCTCTGG TGTCAATTCAC TTTGTTCATGC CTGAGGGTCA GGCCATGCAG GCAAGTGATC  
2400 TGATAGCAAG GTTGGATCTT GATGACCCAT CTTCTGTGAG AAGAGCTGAA CCATTTCATG  
2460 GCACCTTTCC AAAACTTGGA CCTCCTACTG CTATTCTTGG CAAAGTTCAC CAAAAGTTTG  
2520 CTGCAAGTGT GAATTCTGCC CACATGATCC TTGCAGGATA TGAACATAAC ATCAATCATG  
2580 TTGTACAAGA TTTGCTAAAC TGCCTAGACA GCCCTGAGCT CCCTTTCCTA CAGTGGCAAG  
2640 AACTCATGTC CGTTTGGCA ACCCGACTCC CGAAAGATCT TAGGAATGAG TTGGATGCTA  
2700 AGTACAGGA GTATGAGTTG AATGCTGACT TCCGGAAGAG CAAGGATTTC CCTGCCAAGT  
2760 TGCTAAGGGG AGTCATTGAG GCTAATCTTG CATACTGTTT CGAGAAGGAT AGGTTACTA  
2820 GTGAGAGGCT TGTAAGAGCCA CTTATGAGCC TGGTCAAGTC ATATGAGGGT GGAAGAGAAA  
2880 GCCATGCTCG TCGGGTTGTC AAGTCTCTGT TTGAGGAGTA TTTATCTGTT GAAGAAGTCT

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-141-

TCAGCGATGA CATTCACTCT GATGTGATAG AACGTCTACG ACTTCAACAT GCAAAAGACC 2940  
TTGAGAAGGT CGTATATATT GTGTTCTCCC ACCAGGGCGT GAAAAGTAAA AATAAAATTAA 3000  
5 TACTTCGGCT TATGGAAGCA TTGGTCTATC CAAATCCATC TCGGTACAGG GACCAGTTGA 3060  
TTCGCTTTTC TGCCCTTAAC CATAACAGCAT ACTCTGGGCT GGCGCTTAAA GCAAGCCAAC 3120  
TTCTTGAGCA CACTAAATTG AGTGAACCTCC GCACAAGCAT AGCAAGAAGC CTTTCAGAGC 3180  
TGGAGATGTT TACTGAGGAA GGAGAGCGGA TTTCAACACC TAGGAGGAAG ATGGCTATCA 3240  
ATGAAAGGAT GGAAGATTTA GTATGTGCC CCGTTGCAGT TGAAGACGCC CTTGTGGCTT 3300  
15 TGTGTGATCA CAGTGATCCT ACTCTTCAGC GGAGAGTTGT TGAGACATAC ATACGCAGAT 3360  
TGTATCAGCA TTATCTTGTA AGGGCAGTG TCCGGATGCA ATGGCACAGG TCTGGTCTAA 3420  
TTGCTTTATG GGAATTCTCT GAGGAACATA TTGAACAAAG AAATGGGCAA TCTGCGTCAC 3480  
TTCTAAAGCC ACAAGTAGAG GATCCAATTG GCAGGCGATG GGGTGTAAATG GTTGTAATCA 3540  
20 AGTCTCTTCA GCTTCTGTCA ACTGCAATTG AAGCTGCATT AAAGGAGACT TCACATTACG 3600

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GAGCAGGTGT TGGAGGTGTC TCAAATGGTA ATCCTATAAA TTCTAACAGT AGCAATATGC 3660

TGCAATATGC TTTGGTTGGT ATCAACAATC AGATGAGCAC TCTTCAAGAC AGTGGTGATG 3720

5 AGGATCAAGC GCAAGAAAGG ATCAACAAAC TCTCCAAGAT TTTGAAGGAT AACACTATAA 3780

CATCACATCT CAATGGTGCT GGTGTTAGGG TTGTCAAGCTG CATTATCCAA AGAGATGAAG 3840

GGCGTTCACC AATGCGCCAC TCCTTCAAAT GGTCATCTGA CAAGTTATAT TATGAGGAGG 3900

10

ACCCGATGCT CCGCCAATGT GAACCTCCTT TGTCCACCTT CCTTGAATTG GACAAAGTGA 3960

ATTAGAAGG TTACAATGAC GCGAAATACA CCCCATCAG TGATCGCCAG TGGCACATGT 4020

15 ACACACTAGT AAAGAACAAG AAAGATCCGA GATCAAAATGA CCAAAGGATG TTTCTTCGTA 4080

CCATAGTCAG ACAGCCAAGT GTGACCAATG GGT'TTTTGT TGAAGTATT GATAATGAAG 4140

TTCAAGCCTC ATCATCATTC ACATCTAACA GCATACTCAG ATCATTGATG GCAGCGCTAG 4200

20

AAGAAATAGA GTTGCGCGCT CACAGTGAGA CTGGGATGTC AGGCCACTCC CACATGTATC 4260

TGTGCATAAT GAGAGAACAG CGGTGTGTTG ATCTAATTCC ATCTTCAAGG ATGACGAATG 4320

-143-

5 AAGTTGGTCA AGATGAGAAG ACAGCATGCA CATTATTGAA GCATATGGGT ATGATATATA 4380  
TGAGCATGTG GTGTCAGGAT GCATCGCTTT CTGTGTGCCA GTGGGAAGTG AAGCTATGGT 4440  
TGGATTGTGA TGGGCAGGCT AATGGTGCCT GGAGAGTTGT TGTTACCAGT GTAACCTGGGC 4500  
ATACCTGCAC TGTGATATT TACCGAGAAG TGGAGGACCC CAATACACAT CAGCTTTTCT 4560  
ACCGCTCTGC CACACCCACA GCTGGTCCTT TGCATGGCAT TGCATTGCAT GAGCCATACA 4620  
AACCTTTGGA TGCTATTGAC CTGAAACGTG CCGCTGCTAG GAAAAATGAA ACCACATACT 4680  
GCTATGATTT CCCATTGGCA TTTGAAACAG CATTGAAGAA GTCATGGGAA TCTGGTATTT 4740  
15 CACATGTTGC AGAATCTAAC GAGCATAACC AGCGGTATGC TGAAGTGACA GAGCTTATAT 4800  
TTGCTGATTC AACTGGATCA TGGGGTACTC CTTTGGTTCC AGTTGAGCGT CCTCCAGGTA 4860  
GCAACAATTT TGGTGTGTT GCTTGGAACA TGAAGCTCTC CACACCAGAA TTTCAGGCG 4920  
GCCGGGAGAT TATAGTTGTT GCAAATGATG TGACATTAA AGCTGGGTCT TTTGGTCTTA 4980  
GAGAAGATGC ATTCTTTGAT GCTGTCACCA ATCTTGCTTG TGAGAGGAAA ATTCTCTTAA 5040  
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-144-

5100 TTTACTTGTC AGCAACTGCT GGTGCTAGGC TCGGTGTAGC AGAGGAAATA AAGCGTGCT  
5160 TCCATGTTGG ATGGTCTGAT GACCAGAGCC CTGAACGTGG TTTTCACTAC ATTACCTCA  
5220 CTGAACAAGA TTATTCACGT CTAAGCTCTT CAGTTATAGC CCAAGAGCTA AAAGTACCGG  
5280 AAAGCGGAGA AACCAGATGG GTTGTGATA CCATTGTTGG GAAAGAGGAC GGACTTGGTT  
5340 GTGAGAAATCT ACATGGAAGT GGTGCCATTG CCAGTGCCTA CTCTAAGGCA TACAGAGAGA  
5400 CCTTTACTCT GACATTTGTG ACTGGGCGAG CTATTGGAAT TGGGGCTTAT CTGTCTCGGT  
5460 TAGGAATGCG GTGTATACAA CGTCTTGATC AACCAATTAT TTTGACTGGG TATTCTGCAC  
5520 TGAACAAGCT CCTGGGGCGC GAGGTGTATA GCTCTCAGAT GCAACTGGGT GGCCCCAAAA  
5580 TCATGGCTAC AAATGGAGTT GTCCATCTCA CTGTGTCAGA TGATCTTGAA GGTGTTTCTG  
5640 CTATCTTGAA ATGGCTCAGC TATGTTCCCTC CCTATGTTGG CGGTCCTCTT CCTATTGTGA  
5700 AATCTCTTGA TCCACCAGAG AGAGCTGTAA CATATTCCC AGAGAAATCA TGTGATGCCC  
5760 GTGCCGCCAT CTGTGGCATC CAGGACACTC AAGGAGGCAA GTGGTTGGAT GGTATGTTTG

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ACAGAGAAAG CTTTGTGGAA ACATTAGAAG GATGGGCCAA AACTGTTATT ACTGGAAGGG 5820

CAAAGCTAGG TGGGATTCCA GTTGGTATCA TAGCTGTGGA AACCGAGACA GTGATGCAAG 5880

5 TAATCCCTGC TGACCCTGGT CAGCTTGATT CTGCCGAGCG TGTAGTCCCT CAAGCTGGAC 5940

AGGTGTGGTT CCCAGATTCTG GCCGCAAAA CGGCCAGGC ACTGCTGGAT TTCAACCCGTG 6000

AAGAGCTCCC ATTGTTTATA CTTGCTAACT GGAGAGGCTT TTCTGGTGG CAAAGGGATC 6060

10 TGTTTGAAGG AATCCTTCAG GCTGGCTCTA TGATTGTTGA GAATCTGAGG ACGTATAAGC 6120

AGCCTGCCTT TGTGTACATA CCAAGGCTG GAGAGCTGCG TGGAGGTGCA TGGGTTGTGG 6180

15 TGGACAGCAA GATCAATCCT GAGCACATTG AGATGTATGC CGAGAGGACT GCGAGAGGGA 6240

ATGTCCTTGA GGCACCAGGA CTCATTGAGA TCAAGTTCAA GCCAAATGAA CTGGAAGAGA 6300

GTATGCTAAG GCTTGACCCT GAGTTGATCA GCCTCAATGC CAAACTCCTC AAAGAACTA 6360

20 GTGCTAGCCC TAGTCCTTGG GAAACGGCGG CGGCGGCGGA GACCATCAGG AGGAGCATGG 6420

CTGCTCGGAG GAAGCAGCTG ATGCCCATAT ATACTCAGGT TGCCACCCGG TTTGCTGAGT 6480

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5 TGCACGACAC CTCTGCGAGA ATGGCTGCCA AAGGCGTGAT CAGTAAGGTG GTGGACTGGG 6540  
AGGAGTCCCG AGCCTTCTTC TACAGGAGAC TCGGAAGGAG GCTTGCCGAG GACTCGCTCG 6600  
CCAAACAAGT CAGAGAAGCC GCCGGCGAGC AGCAGATGCC CACTCACAGA TCGGCCTTGG 6660  
AATGCATCAA GAAATGGTAC CTGGCCTCTC AGGAGGAGA CGGCGAGAAG TGGGGAGACG 6720  
ATGAAGCCTT CTTCGCCCTGG AAAGATGATC CTGACAAGTA TGGCAAAGTAT CTTGAGGAGC 6780  
TGAAAGCCGA GAGAGCGTCT AACTGTCTGT CGCATCTCGC TGAAACCTCT GATGCCAAGG 6840  
CCTTGCCCCA CGGTCTATCG CTCCTCCTCA GCAAAATGGA TCCTGCAAAG AGGAGGCAGG 6900  
TTATGGATGG CCTCAGGCAG CTTCCTTGTT GATGACTGGC CCACCCCTTG ATAACGGGAG 6960  
CATCCATTCA GCCAGCATAA ACCGGCCTTG CTTGTGCCA CCAAGCAAGT CCTGTCTATG 7020  
GTGGACTGGG TACCAACGGA AGCGCAGACG ACGACAAGCA AATTTTACTT GCGTGGCGAG 7080  
CTACAGGAG GGGAGGTTTT TCAACTGAAG CACATTGTTT GCACATAGGT AGGAGGCATC 7140  
TCATCTCAGG ACAATTTGTA TGTTTATTGT TATTACAGAT AGGTACACAC AAAGCATATG 7200

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TATGCTGGAT AGATATTCCG TGTGAGTTGT TGCAATGCAA GATTCATCAT CTTAATTAC 7260  
 GAGATACGTG TGATGGTCGA TGTGATAGTC CTAGTTTCCT CGGTGGCGAG GAACGCTGAG 7320  
 5 TTTCCTTTTG CTGCAGTTAT GTGATGTATA CCCTGAGAAC 7360

(2) INFORMATION FOR SEQ ID NO: 10:

10 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2257 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

Met Val Glu Ser Asp Gln Ile Asn Gly Arg Met Ser Ser Val Asp Glu  
 1 5 10 15  
 Phe Cys Lys Ala Leu Gly Gly Asp Ser Pro Ile His Ser Val Leu Val  
 20 25 30

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Ala Asn Asn Gly Met Ala Ala Val Lys Phe Met Arg Ser Ile Arg Thr  
35 40 45

Trp Ala Leu Glu Thr Phe Gly Asn Glu Lys Ala Ile Leu Leu Val Ala  
5 50 55 60

Met Ala Thr Pro Glu Asp Leu Arg Ile Asn Ala Glu His Ile Arg Ile  
65 70 75 80

Ala Asp Gln Phe Leu Glu Val Pro Gly Gly Thr Asn Asn Asn Tyr  
85 90 95

Ala Asn Val Gln Leu Ile Val Glu Ile Ala Glu Arg Thr Arg Val Ser  
100 105 110

Ala Val Trp Pro Gly Trp Gly His Ala Ser Glu Asn Pro Glu Leu Pro  
115 120 125

Asp Ala Leu Met Glu Lys Gly Ile Ile Phe Leu Gly Pro Pro Ser Ala  
130 135 140

Ala Met Gly Ala Leu Gly Asp Lys Ile Gly Ser Ser Leu Ile Ala Gln  
145 150 155 160

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Ala Ala Gly Val Pro Thr Leu Pro Trp Ser Gly Ser His Val Lys Val  
165 170 175

Pro Gln Glu Thr Cys His Ser Ile Pro Glu Glu Ile Tyr Lys Asn Ala  
180 185 190

Cys Val Ser Thr Thr Asp Glu Ala Val Ala Ser Cys Gln Val Val Gly  
195 200 205

10 Tyr Pro Ala Met Ile Lys Ala Ser Trp Gly Gly Gly Lys Gly Ile  
210 215 220

Arg Lys Val His Asn Asp Asp Glu Val Arg Ala Leu Phe Lys Gln Val  
225 230 235 240

Gln Gly Glu Val Pro Gly Ser Pro Ile Phe Ile Met Lys Val Ala Ser  
245 250 255

20 Gln Ser Arg His Leu Glu Val Gln Leu Leu Cys Asp Lys His Gly Asn  
260 265 270

Val Ala Ala Leu His Ser Arg Asp Cys Ser Val Gln Arg Arg His Gln  
275 280 285

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Lys Ile Ile Glu Glu Gly Pro Ile Thr Val Ala Pro Pro Glu Thr Ile  
 290 295 300

Lys Glu Leu Glu Gln Ala Ala Arg Arg Leu Ala Lys Cys Val Gln Tyr  
 305 310 315 320

Gln Gly Ala Ala Thr Val Glu Tyr Leu Tyr Ser Met Glu Thr Gly Glu  
 325 330 335

10 Tyr Tyr Phe Leu Glu Leu Asn Pro Arg Leu Gln Val Glu His Pro Val  
 340 345 350

Thr Glu Trp Ile Ala Glu Ile Asn Leu Pro Ala Ser Gln Val Val Val  
 355 360 365

15 Gly Met Gly Ile Pro Leu Tyr Asn Ile Pro Glu Ile Arg Arg Phe Tyr  
 370 375 380

Gly Ile Glu His Gly Gly Gly Tyr His Ala Trp Lys Glu Ile Ser Ala  
 385 390 395 400

20 Val Ala Thr Lys Phe Asp Leu Asp Lys Ala Gln Ser Val Lys Pro Lys  
 405 410 415

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Gly His Cys Val Ala Val Arg Val Thr Ser Glu Asp Pro Asp Asp Gly  
420 425 430

Phe Lys Pro Thr Ser Gly Arg Val Glu Glu Leu Asn Phe Lys Ser Lys  
435 440 445

Pro Asn Val Trp Ala Tyr Phe Ser Val Lys Ser Gly Gly Ala Ile His  
450 455 460

Glu Phe Ser Asp Ser Gln Phe Gly His Val Phe Ala Phe Gly Glu Ser  
465 470 475 480

Arg Ser Leu Ala Ile Ala Asn Met Val Leu Gly Leu Lys Glu Ile Gln  
485 490 495

Ile Arg Gly Glu Ile Arg Thr Asn Val Asp Tyr Thr Val Asp Leu Leu  
500 505 510

Asn Ala Ala Glu Tyr Arg Glu Asn Lys Ile His Thr Gly Trp Leu Asp  
515 520 525

Ser Arg Ile Ala Met Arg Val Arg Ala Glu Arg Pro Pro Trp Tyr Leu  
530 535 540

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-152-

Ser Val Val Gly Gly Ala Leu Tyr Glu Ala Ser Ser Arg Ser Ser Ser  
545 550 555 560

Val Val Thr Asp Tyr Val Gly Tyr Leu Ser Lys Gly Gln Ile Pro Pro  
565 570 575

Lys His Ile Ser Leu Val Asn Leu Thr Val Thr Leu Asn Ile Asp Gly  
580 585 590

10 Gly Lys Tyr Thr Ile Glu Thr Val Arg Gly Gly Pro Arg Ser Tyr Lys  
595 600 605

Leu Arg Ile Asn Glu Ser Glu Val Glu Ala Glu Ile His Ser Leu Arg  
610 615 620

15 Asp Gly Gly Leu Leu Met Gln Leu Asp Gly Asn Ser His Val Ile Tyr  
625 630 635 640

Ala Glu Thr Glu Ala Ala Gly Thr Arg Leu Leu Ile Asn Gly Arg Thr  
645 650 655

Cys Leu Leu Gln Lys Glu His Asp Pro Ser Arg Leu Leu Ala Asp Thr  
660 665 670

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Pro Cys Lys Leu Leu Arg Phe Leu Val Ala Asp Gly Ser His Val Val  
675 680 685

Ala Asp Thr Pro Tyr Ala Glu Val Glu Val Met Lys Met Cys Met Pro  
690 695 700

Leu Leu Leu Pro Ala Ser Gly Val Ile His Phe Val Met Pro Glu Gly  
705 710 715 720

10 Gln Ala Met Gln Ala Ser Asp Leu Ile Ala Arg Leu Asp Leu Asp Asp  
725 730 735

Pro Ser Ser Val Arg Arg Ala Glu Pro Phe His Gly Thr Phe Pro Lys  
740 745 750

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Leu Gly Pro Pro Thr Ala Ile Ser Gly Lys Val His Gln Lys Phe Ala  
755 760 765

Ala Ser Val Asn Ser Ala His Met Ile Leu Ala Gly Tyr Glu His Asn  
770 775 780

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Ile Asn His Val Val Gln Asp Leu Leu Asn Cys Leu Asp Ser Pro Glu  
785 790 795 800

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Leu Pro Phe Leu Gln Trp Gln Glu Leu Met Ser Val Leu Ala Thr Arg  
805 810 815

Leu Pro Lys Asp Leu Arg Asn Glu Leu Asp Ala Lys Tyr Lys Glu Tyr  
820 825 830

Glu Leu Asn Ala Asp Phe Arg Lys Ser Lys Asp Phe Pro Ala Lys Leu  
835 840 845

Leu Arg Gly Val Ile Glu Ala Asn Leu Ala Tyr Cys Ser Glu Lys Asp  
850 855 860

Arg Val Thr Ser Glu Arg Leu Val Glu Pro Leu Met Ser Leu Val Lys  
865 870 875 880

Ser Tyr Glu Gly Arg Glu Ser His Ala Arg Ala Val Val Lys Ser  
885 890 895

Leu Phe Glu Glu Tyr Leu Ser Val Glu Glu Leu Phe Ser Asp Asp Ile  
900 905 910

Gln Ser Asp Val Ile Glu Arg Leu Arg Leu Gln His Ala Lys Asp Leu  
915 920 925



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Glu Lys Val Val Tyr Ile Val Phe Ser His Gln Gly Val Lys Ser Lys  
930 935 940

Asn Lys Leu Ile Leu Arg Leu Met Glu Ala Leu Val Tyr Pro Asn Pro  
945 950 955 960

Ser Ala Tyr Arg Asp Gln Leu Ile Arg Phe Ser Ala Leu Asn His Thr  
965 970 975

10 Ala Tyr Ser Gly Leu Ala Leu Lys Ala Ser Gln Leu Leu Glu His Thr  
980 985 990

Lys Leu Ser Glu Leu Arg Thr Ser Ile Ala Arg Ser Leu Ser Glu Leu  
995 1000 1005

15

Glu Met Phe Thr Glu Glu Gly Glu Arg Ile Ser Thr Pro Arg Arg Lys  
1010 1015 1020

Met Ala Ile Asn Glu Arg Met Glu Asp Leu Val Cys Ala Pro Val Ala  
1025 1030 1035 1040

20

Val Glu Asp Ala Leu Val Ala Leu Phe Asp His Ser Asp Pro Thr Leu  
1045 1050 1055

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Gln Arg Arg Val Val Glu Thr Tyr Ile Arg Arg Leu Tyr Gln His Tyr  
1060 1065 1070

Leu Val Arg Gly Ser Val Arg Met Gln Trp His Arg Ser Gly Leu Ile  
1075 1080 1085

Ala Leu Trp Glu Phe Ser Glu Glu His Ile Glu Gln Arg Asn Gly Gln  
1090 1095 1100

10 Ser Ala Ser Leu Leu Lys Pro Gln Val Glu Asp Pro Ile Gly Arg Arg  
1105 1110 1115 1120

Trp Gly Val Met Val Val Ile Lys Ser Leu Gln Leu Leu Ser Thr Ala  
1125 1130 1135

Ile Glu Ala Ala Leu Lys Glu Thr Ser His Tyr Gly Ala Gly Val Gly  
1140 1145 1150

Gly Val Ser Asn Gly Asn Pro Ile Asn Ser Asn Ser Ser Asn Met Leu  
1155 1160 1165

His Ile Ala Leu Val Gly Ile Asn Asn Gln Met Ser Thr Leu Gln Asp  
1170 1175 1180

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Ser Gly Asp Glu Asp Gln Ala Gln Glu Arg Ile Asn Lys Leu Ser Lys  
1185 1190 1195 1200

Ile Leu Lys Asp Asn Thr Ile Thr Ser His Leu Asn Gly Ala Gly Val  
1205 1210 1215

Arg Val Val Ser Cys Ile Ile Gln Arg Asp Glu Gly Arg Ser Pro Met  
1220 1225 1230

Arg His Ser Phe Lys Trp Ser Ser Asp Lys Leu Tyr Tyr Glu Glu Asp  
1235 1240 1245

Pro Met Leu Arg His Val Glu Pro Pro Leu Ser Thr Phe Leu Glu Leu  
1250 1255 1260

Asp Lys Val Asn Leu Glu Gly Tyr Asn Asp Ala Lys Tyr Thr Pro Ser  
1265 1270 1275 1280

Arg Asp Arg Gln Trp His Met Tyr Thr Leu Val Lys Asn Lys Lys Asp  
1285 1290 1295

Pro Arg Ser Asn Asp Gln Arg Met Phe Leu Arg Thr Ile Val Arg Gln  
1300 1305 1310

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Pro Ser Val Thr Asn Gly Phe Leu Phe Gly Ser Ile Asp Asn Glu Val  
 1315 1320 1325

Gln Ala Ser Ser Phe Thr Ser Asn Ser Ile Leu Arg Ser Leu Met  
 1330 1335 1340

Ala Ala Leu Glu Glu Ile Glu Leu Arg Ala His Ser Glu Thr Gly Met  
 1345 1350 1355 1360

10 Ser Gly His Ser His Met Tyr Leu Cys Ile Met Arg Glu Gln Arg Leu  
 1365 1370 1375

Phe Asp Leu Ile Pro Ser Ser Arg Met Thr Asn Glu Val Gly Gln Asp  
 1380 1385 1390

15 Glu Lys Thr Ala Cys Thr Leu Leu Lys His Met Gly Met Ile Tyr Met  
 1395 1400 1405

Ser Met Trp Cys Gln Asp Ala Ser Leu Ser Val Cys Gln Trp Glu Val  
 1410 1415 1420

Lys Leu Trp Leu Asp Cys Asp Gly Gln Ala Asn Gly Ala Trp Arg Val  
 1425 1430 1435 1440

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Val Val Thr Ser Val Thr Gly His Thr Cys Thr Val Asp Ile Tyr Arg  
1445 1450 1455

Glu Val Glu Asp Pro Asn Thr His Gln Leu Phe Tyr Arg Ser Ala Thr  
1460 1465 1470

Pro Thr Ala Gly Pro Leu His Gly Ile Ala Leu His Glu Pro Tyr Lys  
1475 1480 1485

Pro Leu Asp Ala Ile Asp Leu Lys Arg Ala Ala Arg Lys Asn Glu  
1490 1495 1500

Thr Thr Tyr Cys Tyr Asp Phe Pro Leu Ala Phe Glu Thr Ala Leu Lys  
1505 1510 1515 1520

Lys Ser Trp Glu Ser Gly Ile Ser His Val Ala Glu Ser Asn Glu His  
1525 1530 1535

Asn Gln Arg Tyr Ala Glu Val Thr Glu Leu Ile Phe Ala Asp Ser Thr  
1540 1545 1550

Gly Ser Trp Gly Thr Pro Leu Val Pro Val Glu Arg Pro Pro Gly Ser  
1555 1560 1565

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Asn Asn Phe Gly Val Val Ala Trp Asn Met Lys Leu Ser Thr Pro Glu  
1570 1575 1580

Phe Pro Gly Gly Arg Glu Ile Ile Val Val Ala Asn Asp Val Thr Phe  
1585 1590 1595 1600

Lys Ala Gly Ser Phe Gly Pro Arg Glu Asp Ala Phe Phe Asp Ala Val  
1605 1610 1615

Thr Asn Leu Ala Cys Glu Arg Lys Ile Pro Leu Ile Tyr Leu Ser Ala  
1620 1625 1630

Thr Ala Gly Ala Arg Leu Gly Val Ala Glu Glu Ile Lys Ala Cys Phe  
1635 1640 1645

His Val Gly Trp Ser Asp Asp Gln Ser Pro Glu Arg Gly Phe His Tyr  
1650 1655 1660

Ile Tyr Leu Thr Glu Gln Asp Tyr Ser Arg Leu Ser Ser Ser Val Ile  
1665 1670 1675 1680

Ala His Glu Leu Lys Val Pro Glu Ser Gly Glu Thr Arg Trp Val Val  
1685 1690 1695

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Asp Thr Ile Val Gly Lys Glu Asp Gly Leu Gly Cys Glu Asn Leu His  
 1700 1705 1710

Gly Ser Gly Ala Ile Ala Ser Ala Tyr Ser Lys Ala Tyr Arg Glu Thr  
 1715 1720 1725

Phe Thr Leu Thr Phe Val Thr Gly Arg Ala Ile Gly Ile Gly Ala Tyr  
 1730 1735 1740

Leu Ala Arg Leu Gly Met Arg Cys Ile Gln Arg Leu Asp Gln Pro Ile  
 1745 1750 1755 1760

Ile Leu Thr Gly Tyr Ser Ala Leu Asn Lys Leu Leu Gly Arg Glu Val  
 1765 1770 1775

Tyr Ser Ser Gln Met Gln Leu Gly Gly Pro Lys Ile Met Ala Thr Asn  
 1780 1785 1790

Gly Val Val His Leu Thr Val Ser Asp Asp Leu Glu Gly Val Ser Ala  
 1795 1800 1805

Ile Leu Lys Trp Leu Ser Tyr Val Pro Pro Tyr Val Gly Gly Pro Leu  
 1810 1815 1820

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Pro Ile Val Lys Ser Leu Asp Pro Pro Glu Arg Ala Val Thr Tyr Phe  
 1825 1830 1835 1840

Pro Glu Asn Ser Cys Asp Ala Arg Ala Ile Cys Gly Ile Gln Asp  
 1845 1850 1855

Thr Gln Gly Gly Lys Trp Leu Asp Gly Met Phe Asp Arg Glu Ser Phe  
 1860 1865 1870

Val Glu Thr Leu Glu Gly Trp Ala Lys Thr Val Ile Thr Gly Arg Ala  
 1875 1880 1885

Lys Leu Gly Gly Ile Pro Val Gly Ile Ile Ala Val Glu Thr Glu Thr  
 1890 1895 1900

Val Met Gln Val Ile Pro Ala Asp Pro Gly Gln Leu Asp Ser Ala Glu  
 1905 1910 1915 1920

Arg Val Val Pro Gln Ala Gly Gln Val Trp Phe Pro Asp Ser Ala Ala  
 1925 1930 1935

Lys Thr Gly Gln Ala Leu Leu Asp Phe Asn Arg Glu Glu Leu Pro Leu  
 1940 1945 1950

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Phe Ile Leu Ala Asn Trp Arg Gly Phe Ser Gly Gln Arg Asp Leu  
1955 1960 1965

Phe Glu Gly Ile Leu Gln Ala Gly Ser Met Ile Val Glu Asn Leu Arg  
1970 1975 1980

Thr Tyr Lys Gln Pro Ala Phe Val Tyr Ile Pro Lys Ala Gly Glu Leu  
1985 1990 1995 2000

Arg Gly Gly Ala Trp Val Val Asp Ser Lys Ile Asn Pro Glu His  
2005 2010 2015

Ile Glu Met Tyr Ala Glu Arg Thr Ala Arg Gly Asn Val Leu Glu Ala  
2020 2025 2030

Pro Gly Leu Ile Glu Ile Lys Phe Lys Pro Asn Glu Leu Glu Ser  
2035 2040 2045

Met Leu Arg Leu Asp Pro Glu Leu Ile Ser Leu Asn Ala Lys Leu Leu  
2050 2055 2060

Lys Glu Thr Ser Ala Ser Pro Ser Pro Trp Glu Thr Ala Ala Ala  
2065 2070 2075 2080

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Glu Thr Ile Arg Arg Ser Met Ala Ala Arg Arg Lys Gln Leu Met Pro  
 2085 2090 2095

Ile Tyr Thr Gln Val Ala Thr Arg Phe Ala Glu Leu His Asp Thr Ser  
 2100 2105 2110

5

Ala Arg Met Ala Ala Lys Gly Val Ile Ser Lys Val Val Asp Trp Glu  
 2115 2120 2125

Glu Ser Arg Ala Phe Phe Tyr Arg Arg Leu Arg Arg Arg Leu Ala Glu  
 2130 2135 2140

Asp Ser Leu Ala Lys Gln Val Arg Glu Ala Ala Gly Glu Gln Gln Met  
 2145 2150 2155 2160

15

Pro Thr His Arg Ser Ala Leu Glu Cys Ile Lys Lys Trp Tyr Leu Ala  
 2165 2170 2175

Ser Gln Gly Gly Asp Gly Glu Lys Trp Gly Asp Asp Glu Ala Phe Phe  
 2180 2185 2190

20

Ala Trp Lys Asp Asp Pro Asp Lys Tyr Gly Lys Tyr Leu Glu Glu Leu  
 2195 2200 2205

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Lys Ala Glu Arg Ala Ser Thr Leu Leu Ser His Leu Ala Glu Thr Ser  
2210 2215 2220

Asp Ala Lys Ala Leu Pro Asn Gly Leu Ser Leu Leu Ser Lys Met  
2225 2230 2235 2240

Asp Pro Ala Lys Arg Glu Gln Val Met Asp Gly Leu Arg Gln Leu Leu  
2245 2250 2255

10 Gly

(2) INFORMATION FOR SEQ ID NO: 11:

15 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 984 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

ATGGCTGCAC CTGTCACGAA GAAGCCAATT CTGCTGGAGT TTGAAAAGCC CCTAGTTGAG 60

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CTGGAGGAAC GGATCACGCA AATCCGCACC CTCGCAGCGG ACAACCAGGT GGATGTGAGC 120

GGCCAAATTC AGCAACTGGA AGCCCGGGCG ATTCAACTGC GCGGAGAAAT TTTTAGTAAT 180

CTCTCGCCAG CCCAGCGCAT CCAAGTGGCG CGTCATCCCC GACGTCCGAG TACCTTGGAC 240

TACATCCAAG CGATCAGCGA CGAGTGGATT GAATTACACG GCGATCGCAA CGGTAGTGAT 300

GACCTCGCAC TCGTGGGTGG TGTGGTGGCG CTCGACGGCC AGCCAGTCGT TTTCTTGGGC 360

CACCAAAAGG GCGCGGACAC CAAGGACAAC GTGCTGGCA ACTTCGGGAT GGCTTCACCC 420

GGCGGCTATC GCAAGGCACT GCGTTTGATG GAGCATGCCG ATCGCTTCGG GATGCCGATT 480

CTGACCTTTA TCGATACACC CCGTGTCTTAC GCTGGGGTCA GTGCTGAAGA ACTGGGTCAA 540

GGTGAGGCAA TCGCAGTCAA CCTGCGCGAA ATGTTCCGCT TCTCGGTGCC GATTCTCTGC 600

ACAGTGATTG GCGAAGGCGG TTCTGGGCGG GCCTTGGGCA TTGGCGTCGG CGATCGCCTG 660

CTGATGTTTG AGCATTCCGT CTACACTGTT GCCAGTCCC GAGCCTGCGC ATCAATTCTC 720

TGGCGTGATG CGGGCAAGGC AGCCCAGGCG GCAGAAGCGC TCAAGATTAC GCGCGGAGAC 780

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CTCAAGCAAT TAGGCATCCT TGACGAAATC ATCACCGAAC CTTTGGGCGG TGCCCATTTCT 840  
GCACCGCTGG AAACGGCCCA GAGTTTGGCT CAGGTTTTCG TGCGCCCATCT GAAGGATTTG 900  
5 CAAGCCCTCA GTCCGGCTCA GTTGCGCGAG CAGCGTTATC AAAAGTTTCG CCAGCTCGGG 960  
GTGTTTCTGG AAAGCAGTGA CTAA 984

10 (2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 327 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

20 Met Ala Ala Pro Val Thr Lys Lys Pro Ile Leu Glu Phe Glu Lys  
1 5 10 15

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Pro Leu Val Glu Leu Glu Arg Ile Thr Gln Ile Arg Thr Leu Ala  
20 25 30

Ala Asp Asn Gln Val Asp Val Ser Gly Gln Ile Gln Gln Leu Glu Ala  
35 40 45

Arg Ala Ile Gln Leu Arg Arg Glu Ile Phe Ser Asn Leu Ser Pro Ala  
50 55 60

Gln Arg Ile Gln Val Ala Arg His Pro Arg Arg Pro Ser Thr Leu Asp  
65 70 75 80

Tyr Ile Gln Ala Ile Ser Asp Glu Trp Ile Glu Leu His Gly Asp Arg  
85 90 95

Asn Gly Ser Asp Asp Leu Ala Leu Val Gly Gly Val Gly Ala Leu Asp  
100 105 110

Gly Gln Pro Val Val Phe Leu Gly His Gln Lys Gly Arg Asp Thr Lys  
115 120 125

Asp Asn Val Leu Arg Asn Phe Gly Met Ala Ser Pro Gly Gly Tyr Arg  
130 135 140

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Lys Ala Leu Arg Leu Met Glu His Ala Asp Arg Phe Gly Met Pro Ile  
 145 150 155 160

Leu Thr Phe Ile Asp Thr Pro Gly Ala Tyr Ala Gly Val Ser Ala Glu  
 165 170 175

Glu Leu Gly Gln Gly Glu Ala Ile Ala Val Asn Leu Arg Glu Met Phe  
 180 185 190

10 Arg Phe Ser Val Pro Ile Leu Cys Thr Val Ile Gly Glu Gly Ser  
 195 200 205

Gly Gly Ala Leu Gly Ile Gly Val Gly Asp Arg Leu Leu Met Phe Glu  
 210 215 220

His Ser Val Tyr Thr Val Ala Ser Pro Glu Ala Cys Ala Ser Ile Leu  
 225 230 235 240

Trp Arg Asp Ala Gly Lys Ala Ala Gln Ala Ala Glu Ala Leu Lys Ile  
 245 250 255

Thr Ala Arg Asp Leu Lys Gln Leu Gly Ile Leu Asp Glu Ile Ile Thr  
 260 265 270

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Glu Pro Leu Gly Gly Ala His Ser Ala Pro Leu Glu Thr Ala Gln Ser  
275 280 285

Leu Arg Gln Val Leu Arg His Leu Lys Asp Leu Gln Ala Leu Ser  
290 295 300

Pro Ala Gln Leu Arg Glu Gln Arg Tyr Gln Lys Phe Arg Gln Leu Gly  
305 310 315 320

10 Val Phe Leu Glu Ser Ser Asp  
325

(2) INFORMATION FOR SEQ ID NO: 13:

15

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 22 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

20

(ix) FEATURE:

- (A) NAME/KEY: modified\_base



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(B) LOCATION:one-of(11, 14)  
(D) OTHER INFORMATION:/mod\_base= OTHER  
/note= "N = A, C, G, or T"

5 (ix) FEATURE:  
(A) NAME/KEY: modified\_base  
(B) LOCATION:20  
(D) OTHER INFORMATION:/mod\_base= OTHER  
/note= "R = A or G"

10 (ix) FEATURE:  
(A) NAME/KEY: modified\_base  
(B) LOCATION:17  
(D) OTHER INFORMATION:/mod\_base= OTHER  
15 /note= "H = A, C, or T"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

TCGAATTCGT NATNATHAAR GC

22

20

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:  
25 (A) LENGTH: 22 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

30 (ix) FEATURE:  
(A) NAME/KEY: modified\_base  
(B) LOCATION:one-of(3, 9)  
(D) OTHER INFORMATION:/mod\_base= OTHER  
/note= "Y = C or T"

35

(ix) FEATURE:

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- (A) NAME/KEY: modified\_base
- (B) LOCATION:6
- (D) OTHER INFORMATION:/mod\_base= OTHER  
/note= "N = A, C, G, or T"

5

(ix) FEATURE:

- (A) NAME/KEY: modified\_base
- (B) LOCATION:13
- (D) OTHER INFORMATION:/mod\_base= OTHER  
/note= "K = G or T"

10

(ix) FEATURE:

- (A) NAME/KEY: modified\_base
- (B) LOCATION:12
- (D) OTHER INFORMATION:/mod\_base= OTHER  
/note= "R = A or G"

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

20 GTYCANCTYG TRKGAGATCT CG

22

(2) INFORMATION FOR SEQ ID NO: 15:

25 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

GCTCTAGAAT ACTATTCCT G

21

35

(2) INFORMATION FOR SEQ ID NO: 16:

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## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

5 (D) TOPOLOGY: linear

## (ix) FEATURE:

(A) NAME/KEY: modified\_base

(B) LOCATION: one-of(3, 9)

10 (D) OTHER INFORMATION: /mod\_base= OTHER  
/note= "Y = C or T"

## (ix) FEATURE:

(A) NAME/KEY: modified\_base

15 (B) LOCATION: 6

(D) OTHER INFORMATION: /mod\_base= OTHER  
/note= "N = A, C, G, or T"

## (ix) FEATURE:

20 (A) NAME/KEY: modified\_base

(B) LOCATION: 12

(D) OTHER INFORMATION: /mod\_base= OTHER  
/note= "R = A or G"

## 25 (ix) FEATURE:

(A) NAME/KEY: modified\_base

(B) LOCATION: 13

(D) OTHER INFORMATION: /mod\_base= OTHER  
/note= "K = G or T"

30

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

GTTCANCTYG TRKGAGATCT CG

22

35

## (2) INFORMATION FOR SEQ ID NO: 17:

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## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 23 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ix) FEATURE:

- (A) NAME/KEY: modified\_base
- (B) LOCATION: one-of (9, 11, 14)
- (D) OTHER INFORMATION: /mod\_base= OTHER  
/note= "Y = C or T"

## (ix) FEATURE:

- (A) NAME/KEY: modified\_base
- (B) LOCATION: 18
- (D) OTHER INFORMATION: /mod\_base= OTHER  
/note= "R = A or G"

## (ix) FEATURE:

- (A) NAME/KEY: modified\_base
- (B) LOCATION: 21
- (D) OTHER INFORMATION: /mod\_base= OTHER  
/note= "H = A, C, or T"

## (ix) FEATURE:

- (A) NAME/KEY: modified\_base
- (B) LOCATION: 22
- (D) OTHER INFORMATION: /mod\_base= OTHER  
/note= "M = A or C"

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

GCTCTAGAYT TYAAYGARAT HMG

## (2) INFORMATION FOR SEQ ID NO: 18:

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## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 22 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

5

## (ix) FEATURE:

- (A) NAME/KEY: modified\_base
- (B) LOCATION:2
- (D) OTHER INFORMATION:/mod\_base= OTHER  
/note= "R = A or G"

10

## (ix) FEATURE:

- (A) NAME/KEY: modified\_base
- (B) LOCATION:one-of(3, 13)
- (D) OTHER INFORMATION:/mod\_base= OTHER  
/note= "N = A, C, G, or T"

15

## (ix) FEATURE:

- (A) NAME/KEY: modified\_base
- (B) LOCATION:9
- (D) OTHER INFORMATION:/mod\_base= OTHER  
/note= "Y = C or T"

20

## (ix) FEATURE:

- (A) NAME/KEY: modified\_base
- (B) LOCATION:14
- (D) OTHER INFORMATION:/mod\_base= OTHER  
/note= "W = A or T"

25

30

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

CRNTACTTYT ACNWCTTAAG CT

22

35

## (2) INFORMATION FOR SEQ ID NO: 19:

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## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 398 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

AAAATTATCA TAGTCGCCAA TGACGTTACC TTCAAAGCTG GGTCTTTTGG TCCTAGAGAG 60  
GACGCGTTTT TCCTCGCTGT GACTGAACCC TTGTGCGCGG AGAAGCTTCC CTTGATTTTAC 120  
TTAGCAGCAA ACTCTGGCGC CCGGCTAGGG GTGGCTGAAG AAGTCAAAGC CTGCTTTTAAA 180  
GTTGGATGGT CGGATGAAAGT TTCCCCGGAG AATGGTTTTC AGTATATATA CCTAAGCCCT 240  
GAGGATCAGC AAAGGATTGG ATCATCTGTC ATTGCGCAGC AAATAAAGCT GCCCAGCGGG 300  
GAAACGAGGT GGGTCATTGA TACAATCGTT GGTAAGAAG ATGGTATTGG CGTAGAGAAT 360  
CTAACGGGAA GCGGGGCAAT AGCGGTGCT TACTCGAG 398

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## (2) INFORMATION FOR SEQ ID NO: 20:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 132 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS:  
 (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

1 Lys Ile Ile Ile Val Ala Asn Asp Val Thr Phe Lys Ala Gly Ser Phe 15  
 5  
 10 Gly Pro Arg Glu Asp Ala Phe Phe Leu Ala Val Thr Glu Pro Leu Cys 30  
 15 20 25  
 35 Ala Glu Lys Leu Pro Leu Ile Tyr Leu Ala Ala Asn Ser Gly Ala Arg 45  
 40  
 50 Leu Gly Val Ala Glu Glu Val Lys Ala Cys Phe Lys Val Gly Trp Ser 60  
 55 60

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Asp Glu Val Ser Pro Glu Asn Gly Phe Gln Tyr Ile Tyr Leu Ser Pro  
 65 70 75 80

Glu Asp His Glu Arg Ile Gly Ser Ser Val Ile Ala His Glu Ile Lys  
 85 90 95

Leu Pro Ser Gly Glu Thr Arg Trp Val Ile Asp Thr Ile Val Gly Lys  
 100 105 110

10 Glu Asp Gly Ile Gly Val Glu Asn Leu Thr Gly Ser Gly Ala Ile Ala  
 115 120 125

Gly Ala Tyr Ser  
 130

15

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

20



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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

Pro Leu Asp Phe Asn Glu Ile Arg Gln Leu  
1 5 10

5

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:

- 10 (A) LENGTH: 7 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS:  
(D) TOPOLOGY: linear

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

Leu Asp Phe Asn Glu Ile Arg  
1 5

20

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:

- 25 (A) LENGTH: 23 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ix) FEATURE:

- 30 (A) NAME/KEY: modified\_base  
(B) LOCATION: one-of(9, 11, 14)  
(D) OTHER INFORMATION: /mod\_base= OTHER  
/note= "Y = C or T"

35 (ix) FEATURE:

- (A) NAME/KEY: modified\_base

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(B) LOCATION:18

(D) OTHER INFORMATION:/mod\_base= OTHER  
/note= "R = A or G"

5 (ix) FEATURE:

(A) NAME/KEY: modified\_base

(B) LOCATION:21

(D) OTHER INFORMATION:/mod\_base= OTHER  
/note= "H = A, C, or T"

10

(ix) FEATURE:

(A) NAME/KEY: modified\_base

(B) LOCATION:22

(D) OTHER INFORMATION:/mod\_base= OTHER  
/note= "M = A or C"

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

GCTCTAGAYT TYAAYGARAT HMG

23

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(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:

25

(A) LENGTH: 5 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

Asn Met Lys Met Xaa

1

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35

(2) INFORMATION FOR SEQ ID NO: 25:

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## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 22 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ix) FEATURE:

- (A) NAME/KEY: modified\_base
- (B) LOCATION:2
- (D) OTHER INFORMATION:/mod\_base= OTHER  
/note= "R = A or G"

## (ix) FEATURE:

- (A) NAME/KEY: modified\_base
- (B) LOCATION:one-of(3, 13)
- (D) OTHER INFORMATION:/mod\_base= OTHER  
/note= "N = A, C, G, or T"

## (ix) FEATURE:

- (A) NAME/KEY: modified\_base
- (B) LOCATION:9
- (D) OTHER INFORMATION:/mod\_base= OTHER  
/note= "Y = C or T"

## (ix) FEATURE:

- (A) NAME/KEY: modified\_base
- (B) LOCATION:14
- (D) OTHER INFORMATION:/mod\_base= OTHER  
/note= "W = A or T"

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

CRNTACTTYT ACNWCTTAAG CT

22

## (2) INFORMATION FOR SEQ ID NO: 26:

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## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

5

## (ix) FEATURE:

- (A) NAME/KEY: modified\_base
- (B) LOCATION: one-of (10, 16)
- (D) OTHER INFORMATION: /mod\_base= OTHER  
/note= "N = A, C, G, or T"

10

## (ix) FEATURE:

- (A) NAME/KEY: modified\_base
- (B) LOCATION: 13
- (D) OTHER INFORMATION: /mod\_base= OTHER  
/note= "R = A or G"

15

## (ix) FEATURE:

- (A) NAME/KEY: modified\_base
- (B) LOCATION: one-of (14, 19)
- (D) OTHER INFORMATION: /mod\_base= OTHER  
/note= "Y = C or T"

20

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

GCTCTAGACN CARYTNAAYT T

21

30 (2) INFORMATION FOR SEQ ID NO: 27:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 22 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

35

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## (ix) FEATURE:

- (A) NAME/KEY: modified\_base
  - (B) LOCATION:2
  - (D) OTHER INFORMATION:/mod\_base= OTHER
- 5                    /note= "R = A or G"

## (ix) FEATURE:

- (A) NAME/KEY: modified\_base
  - (B) LOCATION:one-of(3, 13)
  - (D) OTHER INFORMATION:/mod\_base= OTHER
- 10                   /note= "N = A, C, G, or T"

## (ix) FEATURE:

- (A) NAME/KEY: modified\_base
  - (B) LOCATION:9
  - (D) OTHER INFORMATION:/mod\_base= OTHER
- 15                   /note= "Y = C or T"

## (ix) FEATURE:

- (A) NAME/KEY: modified\_base
  - (B) LOCATION:14
  - (D) OTHER INFORMATION:/mod\_base= OTHER
- 20                   /note= "W = A or T"

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

CRNTACTTYG ACNWCTTAAG CT

22

## (2) INFORMATION FOR SEQ ID NO: 28:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- 35

- 184 -

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

GAAGATCTTT ATGGGCGGTA GTATG

25

5

(2) INFORMATION FOR SEQ ID NO: 29:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 23 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

15

GGTCGAAACG GTACAACCTA GGC

23

(2) INFORMATION FOR SEQ ID NO: 30:

20

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 11994 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

25

(ix) FEATURE:

- (A) NAME/KEY: modified\_base
- (B) LOCATION:10357
- (D) OTHER INFORMATION:/mod\_base= OTHER  
/note= "R = A or G"

30

(ix) FEATURE:

- (A) NAME/KEY: modified\_base
- (B) LOCATION:one-of(10198, 10472, 10501, 11698)
- (D) OTHER INFORMATION:/mod\_base= OTHER

35

-185-

/note= "Y = C or T"

## (ix) FEATURE:

(A) NAME/KEY: modified\_base

(B) LOCATION: 10321

(D) OTHER INFORMATION: /mod\_base= OTHER

/note= "K = G or T"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

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GCCCCGCCAA CCAGGGCCAT GCGGCCCAAC TACCCGTCGT CCCCCTCTAG ACCACGCCCC 60

CCACCTGCCC CGCCCCACCC CACCCCCAAC TCCTCCATGA ATGCACGCAT TTCATCGCTC 120

15 CAACCACAA CAGCAGAGCC CAGCACCAGC GGCCTCGGC ACAGGGCGCG CATTATACC 180

ACGCAATTCC ATCTGGATCT CCACCTGGCC GCAGCAGGG TTTCCTCCTC CCTCCCCGCG 240

CGGCATTCCG TCGAACGGCT TGGCGGCGCG CCTCCGGACG GACCCACGGT AAGCTCCCCC 300

TGCCCTTGCT ATGCCCCCTGC TTCTGCAGC ATCTTCCGAT TTTCGCTGGA GCGCTCCGCC 360

TCCGCCTATG CGTGGGGCG ATTGACTGGG CCGGACTTGC CATGGACTCG TACTGACCAG 420

20

-186-

480 TGATGTACTC GCTCGCTAGC CTCTCCGCC AGCCGGCCT CAAATCGAGC GCGCGTAGGC  
540 TGCCCTCCAGG CCCCATCCA AGCAGGCAG CGCAGGGCCT TCCTGCTGAT TCTCTCTCAG  
600 CGCCAGGAGA TCACGGGACC AGATACCACT GCTAGCAGTC GACCCGTGCC GTCGCCGGAT  
660 TGCCGGGTTT GCCCCGTCTG GCATTACGTC GAGCGGGTGG TGGGCGCGCG CGACTGGCCG  
720 GGTTTTGGC ACACCTGTGG CTTACTTCCT TCTGCTGAAT GCCGGAATTC AGTCCATT  
780 CCTCTTTGC TCCTGCTTGG ACTAACCAGT CCCCTAGTGT GGACTACAGC ATTTTITTCG  
840 CGTATTTTAA ATGTGATCTC TGGTCTTGCT CTTCTGGTTC TGCTGGTTGT TGACTAGAAT  
900 TCTGCACTCT CCCATGGCAC TCTTGCCGGA GGAATTTCCC GATTAGCTA GCCGTTAAT  
960 AGTGCCACCA TGTTGTTGTT TTCTGTAGTA CCATTTTAGC ATCTGGTACA GAAAAAGGGC  
1020 ACACACATGC CAAACCGAAA AGAAATATCC CAGTGCTGCA ATTCTACGCT AATCGGACAT  
1080 AAATGATTGA TGCCTAACG GACGGACTTG TTCCTTTGCT TTCCTCCAGCG CTGAAGGTTG  
1140 GAGGGGGCAA TAATGGTGA ATCTGACCAC ATAAACGGGA CGCCCAACAG GATGTCCTCG

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-187-

1200 GTCGATGAAT TCTGTAAAGC GCTCGGGGGT GACTCGCCGA TACACAGCGT GCTGGTTGCC  
1260 AACAAATGGGA TGGCTGCGGT CAAATTCATG CGCAGCATCC GCACCTGGGC CTTGGAGACC  
1320 TTTGGGAACG AGAAGGCCAT TCTCTTGGTG GCTATGGCAA CTCCAGAGGA CCTCAGGATA  
1380 AATGCGGAGC ACATAAGAAAT CGCCGACCAG TTCTTAGAAG TTCCTGCTGG AACGAACAAT  
1440 AACAACTATG CAAATGTACA GCTCATAGTG GAGGTTAGTG CAGTTGATCA TCCTTTTTC  
1500 CCTACTACTT ATGGATTACC ATGTTCAATTA TGCTGGATAC TTGACTAGTT ATTAATCTTT  
1560 CTGATTCACC TGTCCTGTCA CAGATAGCAG AGAGAACTCG GGTTCCTGCA GTTTGGCCTG  
1620 GCTGGGGTCA TGCTTCTGAG AACCCAGAAC TTCCAGACGC GCTCATGGAA AAGGGAATCA  
1680 TTTTCTCTGG GCCACCATCA GCCGCGATGG GGGCACTAGG CGATAAGATT GGTTCCTCTC  
1740 TTATTGCACA AGCAGCAGGA GTTCCAACTC TTCCATGGAG CGGGTCACAT GTATGTATAC  
1800 CTTGTCTCTAT TTCTTTATGG TTTTGCTCTT CTGTTTCTTCT CTCCACCACT GTGTATTCT  
1860 CAAAATAAA TCAATACACG CTGTAGGTGA AAGTCCGCA AGAAACCTGC CACTCAATAC

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CTGAGGAGAT CTATAAGAAC GCTTGTGTTT CAACTACAGA CGAAGCAGTT GCTAGTTGTC 1920

AGGTGGTGG GTATCCTGCA ATGATCAAGG CATCATGGG CGGGGGTGGT AAAGGAATAA 1980

5 GGAAGGTTGG TATTCTTTTC ATCTTTTCAA TTCATCTCTA CCTTAGTTAT ATGGAATGCT 2040

CTACTAGAAA CAATTACATG TAAATTCCAC TGTTCAATTG AAATGAAGTC CAAGTTTCT 2100

GCAATTATTG TATATTAAACC AAAGATGTTT TTTATGTCTAT CAAATGGTTT TATAGGTACA 2160

10 TAATGATGAT GAGGTCAGAG CATTGTTTAA GCAAGTGCAA GGAGAAAGTCC CCGGATCGCC 2220

TATATTTATT ATGAAGGTGG CATCTCAGGT GATACGTGAT AAGCTGATAA CAGCCATTAT 2280

15 TTTCGTGTTG ATCTTTGTGT TACTCATGTT CAGTATTCAG CGAGTGCTTC TTCTGTACTG 2340

ATATAGTTCA TTAGCTAAA ATCTTGCCTT TCTGTACTTT CTTTGTAGAG CCGACATCTA 2400

GAGGTTTCAGT TGCTCTGTGA CAAGCATGGC AACGTGGCAG CACTGCACAG TCGAGACTGT 2460

20 AGTGTTCAAA GAAGGCACCA AAAGGTTAGT TATTCTCCTG AAGCATGGG TTGTTCAATA 2520

TCAGTTTGTG TGAATTAGT CTTAGCCAAA CATTGTGTGA GTGAGTACTG GTAGAAGTTC 2580

-189-

2640 TACAGCTTCA GGGGAATAAA AACTTCATTG GACAATGTAG CAATCATATA GACTGTGTTA  
2700 GCAAAGTGCA AAATGTTGCA GGAGCTATAC CAAATTATG TCGTGGCAAT TTCTTAAATG  
5 2760 GAATCATTTA TTAAGTTAG TTATACTTAT ACTGTACTAA ATAGTTGAAT GTTGCAATTT  
2820 GAATTCAAGA ACAAACTTTT TCTTCCTATA GTGATATATG TGTGTACTT GAAGTTTTTG  
2880 AACTCAGAAT ATTGAAAAGT CTAGTGAAGT TATTACAGAT TATTTGTAA CCAAAAAAAT  
10 2940 TTAAGTAGT CAAGACAGAT AATAGCAGAG AAGTCTTAGC AAAATTATAT TTATTTTACT  
3000 TCTCAGGATA TATATACTTG TGAACACAGAT CATTGAGGAG GGACCAATTA CAGTTGCTCC  
3060 TCCAGAAACA ATTAAGAGC TTGAGCAGGC AGCAAGGCGG CTTGCTAAAT GTGTGCAATA  
15 3120 TCAGGGTGCT GCTACAGTAG AATATCTGTA CAGCATGGA ACAGGCGAAT ACTATTTTCT  
3180 GGAGCTTAAT CCAAGGTTGC AGGTAGAACA CCCTGTGACC GAATGGATTG CTGAAATAAA  
20 3240 CTTACCTGCA TCTCAAGTTG TAGTAGGAAT GGGCATAACCA CTCTACAACA TTCCAGGTAG  
3300 GCCAGTTGTC CAACTTGATG GTTGATGATA TTATCTCTTT CCCCCACAC TAATCAATAT

-190-

3360 AAGGATAACT GCAGAGATCA GACGCTTTTA TGGAATAGAA CATGGAGGTG GCTATCACGC  
3420 TTGGAAGGAA ATATCAGCTG TTGCAACTAA ATTTGATCTG GACAAAGCAC AGTCTGTAAA  
3480 5 GCCAAAGGGT CATTGTGTAG CAGTTAGAGT TACTAGCGAG GATCCAGATG ATGGGTTTAA  
3540 GCCTACAACT GGAAGAGTGG AAGAGCTGAA CTTTAAAAGT AAACCCCAATG TTTGGGCCTA  
3600 TTTCTCTGTT AAGGCAAGTT TGCATCCATG CAGAATGATC TTTGATACCA CATGACATGT  
3660 CACAAACAGCT GCAGCTTATC ATTACCCCTTG AGTTTTCCTG TTTCTTATGT CGATAAATTT  
3720 CCTGGTTAAA AACTGTATCT TGTGTGGCAA ACCTAACCTG AATCATCGTT TTTTGTTCAT  
3780 15 GTCCGGAGGT GCAATTCATG AGTTCTCTGA TTCCCAGTTT GGTAAGTGAT GTGCGTAAAT  
3840 TTCTGTTTCC TCATATATCT CATGATGATG CTTCTCTTAA ACAGCATGCC TTTTTCGCA  
3900 GGTCAATGTTT TTGCTTTTGG GGAATCTAGG TCATTGGCAA TAGCCAATAT GGTACTTGGG  
3960 TTAAAAGAGA TCCAAATTCTG TGGAGAGATA CGCACTAATG TTGACTACAC TGTGGATCTC  
4020 TTGAATGTAA GATAACCCCA CAGTAAACAT GTTCTCTGAT TACATGGTAC ATTTATTAAAG  
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-191-

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5 TGTTAGAGCA GAGAGGCCCC CATGGTACCT TTCAGTTGTT GGTGGAGCTC TATATGTATG 4200  
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10 TGTGTGAACC GATTATGTTG GTTATCTCAG TAAAGGTCAA ATACCACCAA AGGTACATAC 4380  
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15 TCTTTCCGTT CACAGCACAT CTCTCTTGTC AATTGACTG TAACACTGAA TATAGATGGG 4500  
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20 TAATCCAGTT GGTATCCTTG TCACATCTGC TAATTATTAT TTTCTTCTGC AGATTGAGAC 4620  
AGTACGAGGT GGACCCCGTA GCTACAAAAT AAGAATTAAT GAATCAGAGG TTGAAGCAGA 4680  
GATACATTGG CTGCGAGATG GCGGACTCTT AATGCAGGTA GATATATCTA CCAAGTTTTT 4740

-192-

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TGTAATTTT CTAGTTGGAT GGAACAGTC ATGTAATTTA CGCCGAGACA GAAGCTGCTG 4860  
5 GCACGGCTCT TCTAATCAAT GGGAGAACAT GCTTATTACA GGTGAAGATA GCTAGATCTG 4920  
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10 GATCCTTCCA GGTGTGTTGGC TGATACACCA TGCAAGCTTC TTCGGTTTTT GGTCGGGAT 5100  
GGTTCTCATG TGGTTGCTGA TACGCCATAT GCTGAGGTGG AGGTGATGAA AATGTGCATG 5160  
15 CCACTGTTAC TACCGGCCTC TGGTGTCAAT CACTTTGTCA TGCCTGAGGG TCAGGCCCATG 5220  
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TGGTTCATGC TCGTAATATA CGTGCATCTT TTAATAAGTA GCTGAAATGG CTGTCTTTGT 5340  
20 GCAGGCGAGT GATCTGATAG CAAGGTTGGA TCTTGATGAC CCATCTTCTG TGAGAAAGGC 5400  
TGAACCATTT CATGGCACCT TTCCAAAAC TGGACCTCCT ACTGCTATTT CTGGCAAAGT 5460

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5580 TAACATCAAT CATGTAAGGC ACATCAAACACT GTCAGTGTAT ACTTGTCTT CCACCTTTCT  
5640 TTTCCCTTGT CTATCACATT GCCATGGGAA AACAGAGCAT GAGTTCTTCT ACAGAGAGAA  
5700 ACTAACCTCT TAATTGTGAC AACTATACC ATCTTTCTTC AATCAATAAG TTCCTGACTG  
5760 TACCTTTTCT TTCAGGTTGT ACAAGATTTG CTGAACTGCC TAGACAGCCC TGAGCTCCCT  
5820 TTCCTGCAGT GGCAAGAACT CATGTCCGTT TTGGCAACCC GACTCCCAGAA AGATCTTAGG  
5880 AATGAGGTGA ATAAGTATTC AAGTTATATT TTTTATCTTT AGAGTTATTA TTCCATTTTT  
5940 CATTTCCGGCT GCATATCAAA TGGATAACTG ATTTACCTGT TCTCAGTTGG ATGCTAAGTA  
6000 CAAGGAGTAT GAGTTGAATG CTGACTTCCG GAAGAGCAAG GATTTCCCTG CCAAGTTGCT  
6060 AAGGGAGTC ATTGAGGTCA GTTTGAGACT GTTACTTGGC ATCCCTTCCT TTTTATGTG  
6120 TCATGTTGTT TCCTTACAAA GTCATCATTG CAGGCTAATC TTGCATACTG TTCCGAGAAA  
6180 GATAGGGTCA CTAGTGAGAG GCTTGTAGAG CCACTTATGA GTCTGGTCAA GTCATATGAG

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-194-

GGTGAAGAG AAGCCAATGC TCGTGCGGTT GTCAAGTCTC TGTTTGAGGA GTATTATCT 6240  
GTTGAAGAAC TCTTCAGCGA TGACATTCAG GTAACATATT ATAATTGCTT GGAATGGTTT 6300  
5 GATCGATGCT CACTTCTCTGA CCAAAACGTG CTA AACCGTT GTGCTTTTTT GTTTTTATAT 6360  
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10 TGCAAAATTT TCGTGCTGAC AATTGTGTT CTTTGAAGG GTGTGAAAAG TAAAAATAAA 6540  
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15 TTGATTGCGT TCTCTGCCCT GAACCATACA GCATACTCGG GGGTAAAATT GAGTTTGGAT 6660  
GATCTGCATC TATTATTTTT GCACATTGAT ATGATAGTCT AGAAAAATAA AATAAATCTA 6720  
TTGTAATTGA TGCAGCTGGC GCTTAAAGCA AGCCAACTTC TTGAGCACAC CAAATTGAGT 6780  
20 GAACTCCGCA CAAGCATAGC AAGAAGCCTT TCAGAGCTGG AGATGTTTAC TGAGGAAGGA 6840  
GAGCGGATTT CAACACCTAG GAGGAAGATG GCTATCAATG AAAGGATGGA AGATTAGTA 6900



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6960 TGTGCACCGG TTGCAGTTGA AGACGCCCTT GTGGCTTTGT TTGATCACAG TGATCCTACT  
7020 CTTCAGCGGA GAGTAGTCGA GACATACATA CGCAGATTGT ATCAGGTATC ACTGATTTTT  
7080 TTTTTTACTA CACTCTTTCT TGAGACAACT AGAACATTAA CAAATTTATG CCGGCTAACT  
7140 CACAATCACC TTCCAGCATT ATCTTGCAAG GGCAGCGTC CGGATGCAAT GGCATAGGTC  
7200 TGGTCTAATT GCTTTATGGG AATTCTCTGA AGAGCATATT GAACAAAGAA ATGGGCAATC  
7260 TGGGTCACCT CTAAAGCCAC AAGTAGAGGA TCCAATTGGC AGCGATGGG GTGTAATGGT  
7320 TGTAAATCAAG TCTCTTCAGC TTCTGTCAAC TGCAATTGAA GCTGCATTAA AGGAGACTTC  
7380 AACTACGGA GCAGGTGTTG GAAGTGCTC AAATGGTAAT CCTATAAATT TGAACGGCAG  
7440 CAATATGCTG CACATTGCTC TGGTTGGTAT CAACAATCAG ATGAGCACTC TTCAAGACAG  
7500 GTTTGTTTAC ACTCTATTCT TATGTGGTTT GTTGTATTG CACAGGAGAC GAGTGTGATT  
7560 CTGTGAACTG GTCGTAAATT TCATGATTTT TTAGTTACCT CTTCCTCTCT GTTTTCTCTT  
7620 TATAGTGGTG ATGAGGATCA AGCGCAAGAA AGGATCAACA AACTCTCCAA GATTTTGAAG

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GATAACACTA TAACATCACA TCTCAATGGT GCTGGTGTGA GGGTTGTCAG CTGCAATTATC 7680

CAAAGAGATG AAGGGCGTTC ACCAATGCGC CACTCCTTCA AATGGTCATC TGACAAAGTTA 7740

5 TATTATGAGG AGGACCCGAT GCTCCGCCAT GTGGAATCTC CTTTGTCCAC CTTCCCTTGAA 7800

TTGGTATTCA GCTTTTGTGT TGGCTTATGT TCCCTTCAAT AATACCAGTA CCTCTTAACA 7860

GTTTATGTGT AAATACAGGA CAAAGTGAAT TTAGAAGGTT ACAATGACGC GAAATACACC 7920

10 CCATCACGTG ATCGCCAGTG GCACATGTAC ACACTAGTAA AGAACAAAGAA AGATCCGAGA 7980

TCAAAATGACC AAAGGATGTT TCTTCGTACC ATAGTCAGAC AGCCAAGTGT GACCAATGGG 8040

15 TTTTGTGTTG GAAGTATTGA TAATGAAGTT CAAGCCTCGT CATCAATTCAC ATCTAACAGC 8100

ATACTCAGAT CATTGATGGC AGCTCTAGAA GAAATAGAGT TGGGTGCTCA CAGTGAGACT 8160

GGGATGTCAG GCCACTCCCA CATGTATCTG TGCATAATGA GAGAACAAACG GTTGTGTTGAT 8220

20 CTAATTCCAT CTTCAAGGTC AGTCAAAATT TATTTATGTT CTCAACAGAT TATATTGCAT 8280

TAAATATGTT CATAGATGTT CACTTGGTTT TTGCTTCTCA TTATGTTAGG ATGACGAATG 8340

-197-

5 AAGTTGGTCA AGATGAGAAG ACAGCATGCA CACTATTGAA GCATATGGTT ATGAATATAT 8400  
ATGAGCATGT TGGTGTCAAG ATGCATCGCC TTTCCGTGTG CCAGTGGGAA GTGAAGCTAT 8460  
GGTGGATTG TGATGGCAG GCTAATGGTG CTTGGAGAGT TGTGTTACC AGTGTAAC TG 8520  
GCAATACCTG CACTGTTGAT GTAAGTTACC TTAGCTATTG CACTGCTACG CGAGCATTAT 8580  
CATCTACAGT TTTGCAAATA CTACCTCTGA TGGATAAAGC CCCACAGATC ATCAAATATG 8640  
ATTTTGTAG CTTATCTAGT TAGTGAATAG AAAATGTTCA TCACCCCCAT TATGAGTGTA 8700  
ATGGGTAATC TCTCAATTTT TGCCTTTTAAA AGTTCTATTA AACACTACTT AAAAGACTTG 8760  
TAAGTACCAG GTACCATTTT CTCCTTATTG CTCCTATGCT TGAATTATTT TGACTTTTCAG 8820  
ATTTACCGAG AAGTGGAGGA CCCCAATACA CATAAGCTTT TCTATCGCTC TGCCACACCC 8880  
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GACCTGAAAC GTGCCGCTGC TAGGAAAAAT GAAACCACAT ACTGCTATGA TTTCCCAT TG 9000  
GTGCGTTAGC TACATCTCTT TTCTTTTTTT CTCTACAATT GGTTAACATG ATTAAC TAAG 9060

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-198-

ATTGTAATA ATACTCTGTC CGCAGGCATT TGAAACAGCA TTGAAGAAGT CATGGAATC 9120  
TGGTATTICA CATGTTGCAG AATCTAATGA GCATAACCAG CGGTATGCTG AAGTGACAGA 9180  
GCTTATATTT GCTGATTCAA CTGGATCATG GGGTACTCCT TTGGTTCCAG TTGAGCGTCC 9240  
TCCAGGTAGC AACAAATTTG GTGTTGTTGC TTGGAACATG AAGCTCTCCA CACCAGAATT 9300  
TCCAGGTGGC CGGGAGATTA TAGTTGTTGC AAATGATGTG ACATTTAAAG CTGGGTCTTT 9360  
TGGTCCTAGA GAAGATGCAT TCTTTGATGC TGTCACAAAT CTTGCTTGTG AGAGGAAAAT 9420  
TCCTCTAATC TACTTGTGTCAG CAACTGCTGG TGCAAGGCTC GGTGTAGCAG AGGAAATATAA 9480  
GGCATGCTTC CATGTTGGAT GGTCTGATGA CCAGAGCCCT GAACGTGGTT TTCACTACAT 9540  
TTACCTCACT GAACAAGATT ATTCACGTCT AAGCTCTTCA GTTATAGCCC ATGAGCTAAA 9600  
AGTACCAGAA AGCGGAGAAA CCAGATGGGT TGTGTATACC ATTGTTGGGA AAGAGGACGG 9660  
ACTTGTTGT GAGAACTCTAC ATGGAAGTGG TGCCATTGCC AGTGCCTACT CTAAGGCATA 9720  
TAGAGAGACA TTACTCTCTGA CATTTGTGAC TGGCCGAGCT ATTGGAATTG GGGCCTATCT 9780

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-199-

5 TGCTCGGTTA GGAATGCGGT GTATACAACG TCTTGATCAA CCAATTATTT TGA CTGGGTA 9840  
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5 CCCC AAAATC ATGGCTACAA ATGGAGTTGT TCATCTCACT GTGTCAGATG ATCTTGAAGG 9960  
TGTTTCTGCT ATCTTGAAAT GGCTCAGCTA TGTTCCTCCC TATGTTGGTG GTCCCTCTTC 10020  
TATTGTAAAA TCTCTTGATC CACCAGAGAG AGCTGTAACA TACTTTCCAG AGAATTCATG 10080  
TGATGCCCGT GCTGCCATCT GTGGCATTCA GGACACTCAA GGCAAGTGGT TGAGTGGTAT 10140  
GTTTGACAGA GAAAGCTTTG TGGAAACGTT AGAAGGATGG GCCAAAAC TG TTATTACYGG 10200  
15 AAGGGCAAAG CTGGGTGGA TTCCAGTTGG TATCATAGCT GTGGA AACCG AGACAGTGAT 10260  
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KGGACAGGTG TGGTTCCCAG ATTCGGCCCG AAAAACRGCC CAGGCAC TGC TGGATTTC AA 10380  
20 CCGTGAAGAG CTCCCGTTGT TCATACTTGC TAACTGGAGA GGCTTTTCTG GTGGGCAAAG 10440  
GGATCTGTTT GAAGGAATCC TTCAGGCTGG TYCTATGATT GTTGAGAATC TGAGGACGTA 10500

-200-

YAGCAGCCT GCTTTTGTGT ACATACCAA GGCTGGAGAG CTGCGTGGAG GTGCATGGGT 10560  
TGTTGGTGGAC AGCAAGATCA ATCCGGAGCA CATTGAGATG TATGCCGAGA GGA CTGCGGAG 10620  
5 AGGGAATGTC CTTGAGGCAC CGGGA CTCTAT TGAGATCAAA TTCAAGCCAA ATGAATTGGA 10680  
AGAGAGTATG CTAGGGCTGG ACCCTGAGTT GATCAGCCTC AATGCTAAAC TCCTCAAAGA 10740  
AACTAGTGCT AGCCCTAGCC CTTGGGAAC GGCGGCGCG GCAGAGACCA TCAGGAGGAG 10800  
CATGGCTGCT CGGAGGAAGC AGCTGATGCC CATATATACT CAGGTTGCCA CCCGTTTTC 10860  
TGAGTTGCAC GACACCTCCG CAAGAATGGC TGCCAAAGGC GTGATCAGTA AGGTGGTGGA 10920  
15 CTGGGAGGAG TCCCGGGCCT TCTTCTACAG GAGACTGCCA AGGAGGCTTG CCGAGGACTC 10980  
GCTCGCCAAA CAAGTCAGAG AAGCCGCCCG CGAGCAGCAG ATGCCCACTC ACAGATCAGC 11040  
CTTGGAGTGC ATCAGGAAAT GGTACCTGGC CTCTCAAGGA GGAGACGGCG AGAAGTGGGG 11100  
CGATGATGAA GCCTTCTTCA CCTGGAAGA TGATCCTGAC AAGTATGGCA AGTATCTTGA 11160  
GGAGCTGAAA GCCGAGAGAG CGTCTACACT GCTGTCCGAT CTCGCTGAAA CCTCGGACGC 11220  
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CAAGGCCTTG CCCAACGGTC TCTCGCTCCT CCTCAGCAAA GTAAGTTTCT TTTGCTTATT 11280

AGTATTTGTT TGTTCCTTGTA TACATTTCCT AATAAGTTTC TTTTGCTTCT TCTTTTCITT 11340

5 GTTCTTGAT AGTTTTCCTA ATTAAATTCT TTCTGTCCCT AAGTTTCTCT CCTGATACA 11400

TACATTTGAT TGATTGTACA GATGGATCCT GCAAAGAGGG AGCAGGTTAT GGATGGCCTC 11460

AGGCAGCTTC TTGGTTGATT ACTGGCCCCG GCCCTTTGAT AACGCATCCA TTCAGCCAGC 11520

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ATAAATCGGC CTTCCTTGTT GCCACCAAGC AAGTCCTGTC TATGGTGGC TGGGTACCAG 11580

TGGAACAAGC AAATTTTACT TGGTGGCGA GCTACAGAG GGGAGGATT TTCAGCGGAA 11640

15 GAAAACTGAA ACACATTGTT TGCACATAGG TAGGAGGCAT CTCATCTCAG GACAATCYGT 11700

ATGTTTATTG TCATTACAGA TAGGTACACA CAAAGCATAT GTATGCTGGA TAGATAATCG 11760

GTGTGAGTTG TTGCAATGCA AGATTTCATCA TCTTAATTTA CGAGATACGA TGTGATGATC 11820

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GGTCGATGTG GTAGTTGTAG TTTTCCTCAGT GGCAGGGAAT GCCGAGTTTC CTTACGCTGC 11880

AGTTATGTGA TATGTAAACC CTGAGAACTT TGGGGTGATA TGATGGACGT TTTATCAGTT 11940

-202-

11994

TCATGAGAAA TGAATTTGGA GCCGAGGCC CTTACATCAG TTTTTTTTCT TCTA

(2) INFORMATION FOR SEQ ID NO: 31:

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(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2260 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

Met Val Glu Ser Asp Gln Ile Asn Gly Thr Pro Asn Arg Met Ser Ser  
15 1 5 10 15

Val Asp Glu Phe Cys Lys Ala Leu Gly Gly Asp Ser Pro Ile His Ser  
20 25 30

Val Leu Val Ala Asn Asn Gly Met Ala Ala Val Lys Phe Met Arg Ser  
35 40 45



Ile Arg Thr Trp Ala Leu Glu Thr Phe Gly Asn Glu Lys Ala Ile Leu  
50 55 60

Leu	Val	Ala	Ala	Met	Ala	Thr	Pro	Glu	Asp	Leu	Arg	Ile	Asn	Ala	Glu	His
65						70					75					80

[illegible][illegible][illegible]

Glu Leu Pro Asp Ala Leu Met Glu Lys Gly Ile Ile Phe Leu Gly Pro  
130 135 140

Pro Ser Ala Ala Met Gly Ala Leu Gly Asp Lys Ile Gly Ser Ser Leu  
145 150 155 160

[illegible]

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Val Lys Val Pro Gln Glu Thr Cys His Ser Ile Pro Glu Glu Ile Tyr  
180 185 190

Lys Asn Ala Cys Val Ser Thr Thr Asp Glu Ala Val Ala Ser Cys Gln  
195 200 205

Val Val Gly Tyr Pro Ala Met Ile Lys Ala Ser Trp Gly Gly Gly  
210 215 220

Lys Gly Ile Arg Lys Val His Asn Asp Asp Glu Val Arg Ala Leu Phe  
225 230 235 240

Lys Gln Val Gln Gly Glu Val Pro Gly Ser Pro Ile Phe Ile Met Lys  
245 250 255

Val Ala Ser Gln Ser Arg His Leu Glu Val Gln Leu Leu Cys Asp Lys  
260 265 270

His Gly Asn Val Ala Ala Leu His Ser Arg Asp Cys Ser Val Gln Arg  
275 280 285

Arg His Gln Lys Ile Ile Glu Glu Gly Pro Ile Thr Val Ala Pro Pro  
290 295 300

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Glu Thr Ile Lys Glu Leu Glu Gln Ala Ala Arg Arg Leu Ala Lys Cys  
 305 310 315 320

Val Gln Tyr Gln Gly Ala Ala Thr Val Glu Tyr Leu Tyr Ser Met Glu  
 325 330 335

Thr Gly Glu Tyr Tyr Phe Leu Glu Leu Asn Pro Arg Leu Gln Val Glu  
 340 345 350

His Pro Val Thr Glu Trp Ile Ala Glu Ile Asn Leu Pro Ala Ser Gln  
 355 360 365

Val Val Val Gly Met Gly Ile Pro Leu Tyr Asn Ile Pro Glu Ile Arg  
 370 375 380

Arg Phe Tyr Gly Ile Glu His Gly Gly Tyr His Ala Trp Lys Glu  
 385 390 395 400

Ile Ser Ala Val Ala Thr Lys Phe Asp Leu Asp Lys Ala Gln Ser Val  
 405 410 415

Lys Pro Lys Gly His Cys Val Ala Val Arg Val Thr Ser Glu Asp Pro  
 420 425 430

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Asp Asp Gly Phe Lys Pro Thr Ser Gly Arg Val Glu Glu Leu Asn Phe  
 435 440 445

Lys Ser Lys Pro Asn Val Trp Ala Tyr Phe Ser Val Lys Ser Gly Gly  
 450 455 460

Ala Ile His Glu Phe Ser Asp Ser Gln Phe Gly His Val Phe Ala Phe  
 465 470 475 480

Gly Glu Ser Arg Ser Leu Ala Ile Ala Asn Met Val Leu Gly Leu Lys  
 485 490 495

Glu Ile Gln Ile Arg Gly Glu Ile Arg Thr Asn Val Asp Tyr Thr Val  
 500 505 510

Asp Leu Leu Asn Ala Ala Glu Tyr Arg Glu Asn Lys Ile His Thr Gly  
 515 520 525

Trp Leu Asp Ser Arg Ile Ala Met Arg Val Arg Ala Glu Arg Pro Pro  
 530 535 540

Trp Tyr Leu Ser Val Val Gly Gly Ala Leu Tyr Glu Ala Ser Ser Arg  
 545 550 555 560

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Ser Ser Ser Val Val Thr Asp Tyr Val Gly Tyr Leu Ser Lys Gly Gln  
565 570 575

Ile Pro Pro Lys His Ile Ser Leu Val Asn Leu Thr Val Thr Leu Asn  
580 585 590

Ile Asp Gly Ser Lys Tyr Thr Ile Glu Thr Val Arg Gly Gly Pro Arg  
595 600 605

Ser Tyr Lys Leu Arg Ile Asn Glu Ser Glu Val Glu Ala Glu Ile His  
610 615 620

Ser Leu Arg Asp Gly Gly Leu Leu Met Gln Leu Asp Gly Asn Ser His  
625 630 635 640

Val Ile Tyr Ala Glu Thr Glu Ala Ala Gly Thr Arg Leu Leu Ile Asn  
645 650 655

Gly Arg Thr Cys Leu Leu Gln Lys Glu His Asp Pro Ser Arg Leu Leu  
660 665 670

Ala Asp Thr Pro Cys Lys Leu Leu Arg Phe Leu Val Ala Asp Gly Ser  
675 680 685

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His Val Val Ala Asp Thr Pro Tyr Ala Glu Val Glu Val Met Lys Met  
690 695 700

Cys Met Pro Leu Leu Leu Pro Ala Ser Gly Val Ile His Phe Val Met  
705 710 715 720

Pro Glu Gly Gln Ala Met Gln Ala Ser Asp Leu Ile Ala Arg Leu Asp  
725 730 735

Leu Asp Asp Pro Ser Ser Val Arg Arg Ala Glu Pro Phe His Gly Thr  
740 745 750

Phe Pro Lys Leu Gly Pro Pro Thr Ala Ile Ser Gly Lys Val His Gln  
755 760 765

Lys Phe Ala Ala Ser Val Asn Ser Ala His Met Ile Leu Ala Gly Tyr  
770 775 780

Glu His Asn Ile Asn His Val Val Gln Asp Leu Leu Asn Cys Leu Asp  
785 790 795 800

Ser Pro Glu Leu Pro Phe Leu Gln Trp Gln Glu Leu Met Ser Val Leu  
805 810 815

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Ala Thr Arg Leu Pro Lys Asp Leu Arg Asn Glu Leu Asp Ala Lys Tyr  
820 825 830

Lys Glu Tyr Glu Leu Asn Ala Asp Phe Arg Lys Ser Lys Asp Phe Pro  
835 840 845

Ala Lys Leu Leu Arg Gly Val Ile Glu Ala Asn Leu Ala Tyr Cys Ser  
850 855 860

10 Glu Lys Asp Arg Val Thr Ser Glu Arg Leu Val Glu Pro Leu Met Ser  
865 870 875 880

Leu Val Lys Ser Tyr Glu Gly Arg Glu Ser His Ala Arg Ala Val  
885 890 895

15 Val Lys Ser Leu Phe Glu Glu Tyr Leu Ser Val Glu Glu Leu Phe Ser  
900 905 910

20 Asp Asp Ile Gln Ser Asp Val Ile Glu Arg Leu Arg Leu Gln His Ala  
915 920 925

Lys Asp Leu Glu Lys Val Val Tyr Ile Val Phe Ser His Gln Gly Val  
930 935 940

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Lys Ser Lys Asn Lys Leu Ile Leu Arg Leu Met Glu Ala Leu Val Tyr  
945 950 955 960

Pro Asn Pro Ser Ala Tyr Arg Asp Gln Leu Ile Arg Phe Ser Ala Leu  
965 970 975

5

Asn His Thr Ala Tyr Ser Gly Leu Ala Leu Lys Ala Ser Gln Leu Leu  
980 985 990

Glu His Thr Lys Leu Ser Glu Leu Arg Thr Ser Ile Ala Arg Ser Leu  
995 1000 1005

10

Ser Glu Leu Glu Met Phe Thr Glu Glu Gly Glu Arg Ile Ser Thr Pro  
1010 1015 1020

15

Arg Arg Lys Met Ala Ile Asn Glu Arg Met Glu Asp Leu Val Cys Ala  
1025 1030 1035 1040

Pro Val Ala Val Glu Asp Ala Leu Val Ala Leu Phe Asp His Ser Asp  
1045 1050 1055

20

Pro Thr Leu Gln Arg Arg Val Val Glu Thr Tyr Ile Arg Arg Leu Tyr  
1060 1065 1070



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Gln His Tyr Leu Ala Arg Gly Ser Val Arg Met Gln Trp His Arg Ser  
1075 1080 1085

Gly Leu Ile Ala Leu Trp Glu Phe Ser Glu Glu His Ile Glu Gln Arg  
1090 1095 1100

Asn Gly Gln Ser Ala Ser Leu Leu Lys Pro Gln Val Glu Asp Pro Ile  
1105 1110 1115 1120

10 Gly Arg Arg Trp Gly Val Met Val Ile Lys Ser Leu Gln Leu Leu  
1125 1130 1135

Ser Thr Ala Ile Glu Ala Ala Leu Lys Glu Thr Ser His Tyr Gly Ala  
1140 1145 1150

15 Gly Val Gly Ser Val Ser Asn Gly Asn Pro Ile Asn Leu Asn Gly Ser  
1155 1160 1165

20 Asn Met Leu His Ile Ala Leu Val Gly Ile Asn Asn Gln Met Ser Thr  
1170 1175 1180

Leu Gln Asp Ser Gly Asp Glu Asp Gln Ala Gln Glu Arg Ile Asn Lys  
1185 1190 1195 1200

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Leu Ser Lys Ile Leu Lys Asp Asn Thr Ile Thr Ser His Leu Asn Gly  
 1205 1210 1215

Ala Gly Val Arg Val Val Ser Cys Ile Ile Gln Arg Asp Glu Gly Arg  
 1220 1225 1230

5

Ser Pro Met Arg His Ser Phe Lys Trp Ser Ser Asp Lys Leu Tyr Tyr  
 1235 1240 1245

Glu Glu Asp Pro Met Leu Arg His Val Glu Ser Pro Leu Ser Thr Phe  
 1250 1255 1260

Leu Glu Leu Asp Lys Val Asn Leu Glu Gly Tyr Asn Asp Ala Lys Tyr  
 1265 1270 1275 1280

15

Thr Pro Ser Arg Asp Arg Gln Trp His Met Tyr Thr Leu Val Lys Asn  
 1285 1290 1295

Lys Lys Asp Pro Arg Ser Asn Asp Gln Arg Met Phe Leu Arg Thr Ile  
 1300 1305 1310

20

Val Arg Gln Pro Ser Val Thr Asn Gly Phe Leu Phe Gly Ser Ile Asp  
 1315 1320 1325

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Asn Glu Val Gln Ala Ser Ser Ser Phe Thr Ser Asn Ser Ile Leu Arg  
 1330 1335 1340  
 Ser Leu Met Ala Ala Leu Glu Glu Ile Glu Leu Arg Ala His Ser Glu  
 1345 1350 1355 1360  
 Thr Gly Met Ser Gly His Ser His Met Tyr Leu Cys Ile Met Arg Glu  
 1365 1370 1375  
 Gln Arg Leu Phe Asp Leu Ile Pro Ser Ser Arg Met Thr Asn Glu Val  
 1380 1385 1390  
 Gly Gln Asp Glu Lys Thr Ala Cys Thr Leu Leu Lys His Met Val Met  
 1395 1400 1405  
 Asn Ile Tyr Glu His Val Gly Val Arg Met His Arg Leu Ser Val Cys  
 1410 1415 1420  
 Gln Trp Glu Val Lys Leu Trp Leu Asp Cys Asp Gly Gln Ala Asn Gly  
 1425 1430 1435 1440  
 Ala Trp Arg Val Val Val Thr Ser Val Thr Gly Asn Thr Cys Thr Val  
 1445 1450 1455

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Asp Ile Tyr Arg Glu Val Glu Asp Pro Asn Thr His Lys Leu Phe Tyr  
1460 1465 1470

Arg Ser Ala Thr Pro Thr Ala Gly Pro Leu His Gly Ile Ala Leu His  
1475 1480 1485

Glu Pro Tyr Lys Pro Leu Asp Ala Ile Asp Leu Lys Arg Ala Ala Ala  
1490 1495 1500

10 Arg Lys Asn Glu Thr Thr Tyr Cys Tyr Asp Phe Pro Leu Ala Phe Glu  
1505 1510 1515 1520

Thr Ala Leu Lys Lys Ser Trp Glu Ser Gly Ile Ser His Val Ala Glu  
1525 1530 1535

15 Ser Asn Glu His Asn Gln Arg Tyr Ala Glu Val Thr Glu Leu Ile Phe  
1540 1545 1550

Ala Asp Ser Thr Gly Ser Trp Gly Thr Pro Leu Val Pro Val Glu Arg  
1555 1560 1565

20 Pro Pro Gly Ser Asn Asn Phe Gly Val Val Ala Trp Asn Met Lys Leu  
1570 1575 1580

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Ser Thr Pro Glu Phe Pro Gly Gly Arg Glu Ile Ile Val Val Ala Asn  
1585 1590 1595 1600

Asp Val Thr Phe Lys Ala Gly Ser Phe Gly Pro Arg Glu Asp Ala Phe  
1605 1610 1615

Phe Asp Ala Val Thr Asn Leu Ala Cys Glu Arg Lys Ile Pro Leu Ile  
1620 1625 1630

Tyr Leu Ser Ala Thr Ala Gly Ala Arg Leu Gly Val Ala Glu Glu Ile  
1635 1640 1645

Lys Ala Cys Phe His Val Gly Trp Ser Asp Asp Gln Ser Pro Glu Arg  
1650 1655 1660

Gly Phe His Tyr Ile Tyr Leu Thr Glu Gln Asp Tyr Ser Arg Leu Ser  
1665 1670 1675 1680

Ser Ser Val Ile Ala His Glu Leu Lys Val Pro Glu Ser Gly Glu Thr  
1685 1690 1695

Arg Trp Val Val Asp Thr Ile Val Gly Lys Glu Asp Gly Leu Gly Cys  
1700 1705 1710

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Glu Asn Leu His Gly Ser Gly Ala Ile Ala Ser Ala Tyr Ser Lys Ala  
1715 1720 1725

Tyr Arg Glu Thr Phe Thr Leu Thr Phe Val Thr Gly Arg Ala Ile Gly  
1730 1735 1740

Ile Gly Ala Tyr Leu Ala Arg Leu Gly Met Arg Cys Ile Gln Arg Leu  
1745 1750 1755 1760

Asp Gln Pro Ile Ile Leu Thr Gly Tyr Ser Ala Leu Asn Lys Leu Leu  
1765 1770 1775

Gly Arg Glu Val Tyr Ser Ser Gln Met Gln Leu Gly Gly Pro Lys Ile  
1780 1785 1790

Met Ala Thr Asn Gly Val Val His Leu Thr Val Ser Asp Asp Leu Glu  
1795 1800 1805

Gly Val Ser Ala Ile Leu Lys Trp Leu Ser Tyr Val Pro Pro Tyr Val  
1810 1815 1820

Gly Gly Pro Leu Pro Ile Val Lys Ser Leu Asp Pro Pro Glu Arg Ala  
1825 1830 1835 1840

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Val Thr Tyr Phe Pro Glu Asn Ser Cys Asp Ala Arg Ala Ala Ile Cys  
1845 1850 1855

Gly Ile Gln Asp Thr Gln Gly Lys Trp Leu Ser Gly Met Phe Asp Arg  
1860 1865 1870

Glu Ser Phe Val Glu Thr Leu Glu Gly Trp Ala Lys Thr Val Ile Thr  
1875 1880 1885

10 Gly Arg Ala Lys Leu Gly Gly Ile Pro Val Gly Ile Ile Ala Val Glu  
1890 1895 1900

Thr Glu Thr Val Met Gln Val Ile Pro Ala Asp Pro Gly Gln Leu Asp  
1905 1910 1915 1920

Ser Ala Glu Arg Val Val Pro Gln Ala Gly Gln Val Trp Phe Pro Asp  
1925 1930 1935

Ser Ala Ala Lys Thr Ala Gln Ala Leu Leu Asp Phe Asn Arg Glu Glu  
1940 1945 1950

Leu Pro Leu Phe Ile Leu Ala Asn Trp Arg Gly Phe Ser Gly Gly Gln  
1955 1960 1965

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-218-

Arg Asp Leu Phe Glu Gly Ile Leu Gln Ala Gly Xaa Met Ile Val Glu  
 1970 1975 1980  
 5

Asn Leu Arg Thr Tyr Lys Gln Pro Ala Phe Val Tyr Ile Pro Lys Ala  
 1985 1990 1995 2000  
 Gly Glu Leu Arg Gly Gly Ala Trp Val Val Asp Ser Lys Ile Asn  
 2005 2010 2015

10 Pro Glu His Ile Glu Met Tyr Ala Glu Arg Thr Ala Arg Gly Asn Val  
 2020 2025 2030  
 Leu Glu Ala Pro Gly Leu Ile Glu Ile Lys Phe Lys Pro Asn Glu Leu  
 2035 2040 2045

15 Glu Glu Ser Met Leu Gly Leu Asp Pro Glu Leu Ile Ser Leu Asn Ala  
 2050 2055 2060  
 Lys Leu Leu Lys Glu Thr Ser Ala Ser Pro Ser Pro Trp Glu Thr Ala  
 2065 2070 2075 2080  
 Ala Ala Ala Glu Thr Ile Arg Arg Ser Met Ala Ala Arg Arg Lys Gln  
 2085 2090 2095

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Leu Met Pro Ile Tyr Thr Gln Val Ala Thr Arg Phe Ala Glu Leu His  
2100 2105 2110

Asp Thr Ser Ala Arg Met Ala Ala Lys Gly Val Ile Ser Lys Val Val  
2115 2120 2125

Asp Trp Glu Glu Ser Arg Ala Phe Phe Tyr Arg Arg Leu Arg Arg Arg  
2130 2135 2140

Leu Ala Glu Asp Ser Leu Ala Lys Gln Val Arg Glu Ala Ala Gly Glu  
2145 2150 2155 2160

Gln Gln Met Pro Thr His Arg Ser Ala Leu Glu Cys Ile Arg Lys Trp  
2165 2170 2175

Tyr Leu Ala Ser Gln Gly Gly Asp Gly Glu Lys Trp Gly Asp Asp Glu  
2180 2185 2190

Ala Phe Phe Thr Trp Lys Asp Asp Pro Asp Lys Tyr Gly Lys Tyr Leu  
2195 2200 2205

Glu Glu Leu Lys Ala Glu Arg Ala Ser Thr Leu Ser His Leu Ala  
2210 2215 2220

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Glu Thr Ser Asp Ala Lys Ala Leu Pro Asn Gly Leu Ser Leu Leu Leu  
2225 2230 2235 2240

Ser Lys Met Asp Pro Ala Lys Arg Glu Gln Val Met Asp Gly Leu Arg  
2245 2250 2255

Gln Leu Leu Gly  
2260

10 (2) INFORMATION FOR SEQ ID NO: 32:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3319 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

20 GGATCCTCTT GAGCTTCTTC AGCAGAGATA CAGTTGACAT GGCCACGTGC AGTGGTGGCT 60

GGCTTGGCGT AGAACACTTT CCCTGTGGGC TTGCCACGGC CAACAGCTTT TCCAGATTGG 120

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TTGGGTTGG TCTGGGGACA CTGCGGCAGA TAGTGGCCCG GTTCCCCACA TTAAAGCAA 180  
GTCACCGAGC TGGTACGTGG AGGAGCATTG TTGGTTGGAC CACCATAGGG CTTGGCTGGT 240  
5 GTAAACTGTT GGGCGGGGCA AGGCGCCTGA AAGATGGCC TCGGTGTGAA CTTGGGTGGC 300  
AGGCAGTGT TAGGCACCCA CACACGGGCG TTCTGAGGAC CAGCACCGGA TGAGGAACCC 360  
ATGTCACGGC CATGCTTGGG TGTTGCTTCA TAATCAGTCT GACCAGACTC AGCATTGATG 420  
10 GCTTTGTATA CAAGCTTCTG AAAAGAATG CACTCATGCA GACGGAGGTC GCGGCGAAGC 480  
TCAGGACTAA GTCCCCATG GAACCTTGCT TGCTTCTTGG CTTCAGTAGA GACTTCCTCA 540  
15 GTTGCAATC GTGCAAGGTT ACCGAACTCC CTACTGTAAG CATCCACAGA AAGTCGACCT 600  
TGAGTGAAAC TGCAGAACTC CTCATGTTTA CGGTCCATGA GACCCTTCGG AATGTGATGT 660  
20 TCAGGCAAAG CCTCGCTGAA TTCAGCCCCAG GTAGTGACAT GGCCCGCTGG GCGCATAGCT 720  
CCATAGTTCT CCCACCATAG ACTGGCGGGG CCTTCAAGAT GATATGCAGC AAAGGTGACC 780  
TTATCAGCCT CAGCTACTAG CGCAGAATGC AGTTTGTGAG TAATACTGCG AAGCCAGTCA 840

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TCGGCGTCGA GAGGCTCGAC GGAGTGGTGG AAAGTGGGTG GATGTAAC TT GATGAAATCA 900

CTGAGTGACA CCAAGTTATT CCTCTGATGG TGTGCCATGT TTTGCTCGAT GCGCTCCAAC 960

5 AAACGGTTAG TCTCCCGCTT GTTCTCTCTG GCTTCCAGCA TAACTTCGGC CAGAGAGGGA 1020

GGGTGAGGCA GGTGTGTTC CCCTCTCTG CTGCTCTGG CCTGCTCTGG GGGAGCAGGG 1080

TTGGTGCGGG TGTTAACCAT CCTAGGAAAA CAAAACAATA GTTTAGTCCA GGATGATAGG 1140

10 ATTCTGACAT AGAACGAAGA ATGTAATGGA TAACTTGGA TGTAAAGATGA CCATCCGTAT 1200

GACATGGTAG ATACAGAAAC TGCTTCTTTT ATTCCATCGT CATAACACACC ATACAAGGTT 1260

15 TAGTACAGAA CCAAAACAAG TACTACTACG GTGAAAAGAG GATTACATCT CATCGGAGGC 1320

ATTCCGAGCT CCTATACATT ATTTTCTTAC ACCTCCGGAA GCGGGTACAA GCTAAGTCAT 1380

ATCCCACGAG TCACGCAGGA CGGTGGATGA TACAGCTAGT ACGATACTAG TGATACTACT 1440

20 ACTAACTCAG ACAACTCCGT AGTAGTCTTC ATATAAGTCA CCTCCATAGC CTGGAAGCTC 1500

AACGTGATCG TGATCCTTCT TTTTCTGTTCTG TCGTAGGGGC TGTTGGGAGG GATTAAATCA 1560

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1620 TTCGCTCCAG AACTGATGAC ATCGCGTTAT GCACGTCCTA TTTAAAATCA CAGACATGAG  
1680 TGAATAAAGT ATGATATGAC GTTATGGCGC AACGGACAAC ATGGGAACAT GACATGTTTC  
1740 ATCTCCCACA CATAACACGA AAACCAGAAC AAAACACCCC GCGACTACGA TTGGAGATGT  
1800 AGGCATCAA GCGTCGAGA CCTATGCCAA GCACACCATC CATCTGTGAC CATGAAGCAC  
1860 AACTATTTCAT CTTCCACCAG CCCC GCCTCC ATGAATGTTG GACTAGAATG TGAATGTGTA  
1920 CTGCCGCGTG CCGGTGTGTC CGTTTGCCTC GCGGGAACAC CACCAGCCCG GTACAGCAAG  
1980 CGATTGTGA CCGTCAACTA AATTGTGAAT CGTTGGCGCA TAATCATTTG AATATGCATG  
2040 TCTCCGTTAC AAGGCACGGA CAATTAGCTA GACAACACAC CCATGATGCA ATTAGCTAGA  
2100 CAATTAGCTA GACAACACAC CCACGGACAA TTAGCACCGA CGACTACGGG ACGGCCGGAC  
2160 GGTGACGGGG ACGTGGACGA AGCCGAGCGG AGCAGGCCAC CGGAGCGGAG GGAGCGAGCT  
2220 GAGCACATCG AGTCCAGGGC AGACACGCCG GAGAGACAGG TGCAACGACG CACCCATCCG  
2280 TCCATCCGCC CGCCCAACCA GGGCCATGCG GCCCAACTAC CCGTCGTCCC CGTCTAGACC

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ACGCCCCCA CCTGCCCCG CCCACCCAC CCCCAACTCC TCCATGAATG CACGCATTTC 2340  
ATCGCTCCAA CCACAAGCA GCAGCCCCAG CACCAGCGGC CTCGGCGACG CGGCGCGCAT 2400  
5 TTATACCAG CAATTCCATC TGGATCTCCA CCTGGCCGCA GCACGGGTTT CCTCCTCCCT 2460  
CCCCGCGGG CATTCCGTCG AACGGCTTGG CGGCGGCGCT CCGGACGGAC CCACGGTAAG 2520  
CTCCCCCTGC CCTTGCTATG CCCCTGCTTC TGCAGGCATC TTCCGATTTT CGCTGGAGCG 2580  
CTCCGGCTCC GCCTATGCGT GCGGGCGATT GACTGGGCGG GACTTGCCAT GGAATCGTAC 2640  
TGACCAGTGA TGTA CTGCT CGCTAGCCTC TCCGCCACG CCGGCCTCAA ATCGAGCGCG 2700  
15 CGTAGGCTGC CTCCAGGCC CAATCCAAGC AGCGCAGCGC AGGGCCTTCC TGCTGATTCT 2760  
CTCTCAGCGC CAGGAGATCA CGGACCAGA TACCACTGCT AGCAGTCGAC CCGTGCCGTC 2820  
GCCGGATTGC CGGGTTCCG CCTCTGGCA TTACGTCGAG CGGGTGGTGG GCGCGCGCGA 2880  
20 CTGGCCGGGT TTTGGGCACA CTTGTGCTT ACTTCCTTCT GCTGAATGCC GGAATTCAAG 2940  
TCCATTTCCTCCTCTTGCTCC TGCTTGGACT AACCAGTCCC CTAGTGTGGA CTACAGCATT 3000

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TTTTTCGGGT ATTTTAAATG TGATCTCTGG TCTTGCTCTT CTGGTTCTGC TGGTGTGGA 3060  
CTAGAATTCT GCACTCTCCC ATGGCACTCT TGCCGGAGGA ATTTCCCGAT TTAGCTAGCC 3120  
5 GTTAATTAGT GCCACCATGT TGTTGTTTTT TGTAGTACCA TTTTAGCATC TGGTACAGAA 3180  
AAAGGGCACA CACATGCCAA ACCGAAAAGA AATATCCCAG TGCTGCAATT CTACGCTAAT 3240  
CGGACATAAA TGATTGATGC GCTAACGGAC GGACTTGTTT TTTTGCTTTT CCCAGCGCTG 3300  
10 AAGTTGGAG GGGGCAATA 3319

(2) INFORMATION FOR SEQ ID NO: 33:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3368 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

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TACTGCGCG CGGACGCGG GTAGGCATGG GCGTATGCAT CCTACTGTTT CTGTGGATC 60  
TACTGCGCG CGGACGCGG GTAGGCATGG GCGTGTGCG GCGCCTGCAG CACGCTGACG 120  
5 CAGCTGGACA GGGACTCGGA GCCCATGGAC AACGTCAGG GGTGCAGGAG GGCACCAGGG 180  
TTGCCGTGGT TGGCCGGCAC GATAGCCCC TCGACGTCCT CGTCGTCGCC GGTGTCCACG 240  
TCGTAGACGT CTCCTGCACG CGCCATCCAT GGCCTCCTGC CGCCATGCGC CGCCGCGACA 300  
10 GTGCCCATGG CCTCCTGCCG CCGTGCGCG TCCTCATGAA CTA CTGCGCGC CGCTCGCCGT 360  
GGCCACTTGC CGGTCCGCTG AGTCCGGCCC GGTCTGGAGA GCCGCCGGTC TGGTCAGTGG 420  
15 TCACGGAAAA CAGGACTGCC AGGCTGGTCG GATCGGCCCC GACAGTTTCC ACCCTGATGA 480  
TCAGGCAGG CGTCGAATCA GACGCCGCGG CAACTCCGAT GTCCCAGACG GCGGCGACAG 540  
AGGTGGTGTG TAGCGTATCC TTGGCAGATG CAACGGCGGA TAGTAAGAG GATTAGAGAA 600  
20 GATATGTTTT CAGCCGAGAA AGAACAGGAA GGGATGACGA CGTAGATAGA CGGCACGGGG 660  
AGGGATGAAG GGCATGTTTT GGATGCCGAT AGCATGAGAT GCGGGGCGGG AAGAGATCAA 720



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TTAGGTTGAG TGGCTTCCTA TTTTAGCTGA TAATAATAAT TAGATGACAA TTATATATGG 780  
TAGGAGTAAT AAGTTTTTTA ATAGGATGGA TTTGTCTGAG ATTAGTTTCC TAATAGGATG 840  
5 GATGCACTCT GATTTAGTTT CATAGAAAAG GATGCACCGC GATTATATAG TTTCCCTAATT 900  
GCCCAGGCGT GGAGTTTCAT ATTTTCCTCC ACAGTGGAGT ACGGCCAGTC AATGTAAATT 960  
GCTAAGTGCA CACAGAAAAT GGTTAGGTT AAGGCTAACC GTTAGATTGA TTTTAGTGGG 1020  
10 CCTAATCGTG CGGTGGTATT GGATCTGTGT ACGCTTTGTG GGGTGTGCTT AAAAAAGTTC 1080  
TTATTTGATT GTTTAATAGT AGTATAGATA AAAAAGGCAC GCCTTCGTTA ACGCGCGTAG 1140  
15 AAAAAATATT TGAATCACAA ACAAGAGCTA ACAAAGCAT GATATGCCCT TGTGGCAAAA 1200  
CCGGTGACAC GGGAGTACAA CATGTTTCAC CACCAACACG TCACCCGAGA AACGGAATAA 1260  
ACACCCCGCA GTATGTTTGA GCGTTGGCA TCAAAAGCGT TGGGACCTAT GCTAGGCACA 1320  
20 ACATCCATCC GTACGGCGA AGCGCAACTA TTGTCTTCAA GGGGAAATGG AATCGACTCC 1380  
GCACCAACGG GAGCGGAGGG AGTCTACATC ACACCCGTCA CGTGTCCCGG CCCCCTAAAT 1440

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GCACGACTAG AAGGTGCACC ATTGCATCCT CAAAAAAGAA AAAAAAAGC GAATCAACCT 1500  
GTGGTTGGTT GGTAGAGG ACTGTGGTAT CCCAGCCCCA CCATGGTTCA AATCCTGGTG 1560  
CTCGCATTTA TTCTGGATT TATTTAGGA TTCCGGCGA TCGCATTTCA GTGGGAGGTT 1620  
CATAGGGATG AGTGATATACG CGTGATATATG AGCGCTTGCG TCTGTACTGT GTTAAAAAAA 1680  
AAGAAAAAAA AAGATTATGT ACCATTGGCG GTGTATGTCC ATACACTTGA GCCGATTAGC 1740  
TAGAGAACAG GGTCAATGATG CAGTCCGAGT TACGGTAACG AACAAACGGG AGTCAACAAG 1800  
CGGGCACAAG ACGCCGTGGT GGCTTGGCCG ACGACTACGG GACGGCCGGA CGGGTCGGGG 1860  
ACGTGAGCGA AGCCGAAGGG AGCAGCCAC CGGAGCGGAA GGAGCGAGCA CATCGAAGGC 1920  
GTTGGGGCCC TACCTACACA CACGCCGGAG AGACAGGTGC AACGACACAC CAATCCGTCC 1980  
AACCAGGGCG ATGAGGCCCA ACAACCTGTC GTCGACTCCT CCCCCTCTCC ACCTCCACCA 2040  
CACCCCCAC CTGCCCCGCC CCACCCCACC CCACCCCCAA CTCCTCCAAG AATGCACGCA 2100  
TTTCATCGCT CCTACCACAA CGCAGCAGCA CCAGCGGCCT CGGCGACGCG CCGCGCATTT 2160

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ATAGCAAGCA ATTCCTCGTT GCCTCCGCCT CCGCCGCCGC TGCCTCTCCT GGATCTCCAT 2220  
CTGGCCGCAG CACGGCCTTC TTCCTCCTTC CTCCTCCGC GGCATTCCGT CGAACGGCTT 2280  
5 CGCGGCGGG CTCGGGCCGA ACCGACGGTA CGGCCCTGC CCGTCCCCC TGCCCCCGCC 2340  
GTGCCCTGC TTCGCCCCC CTCCTCCGGT TTTCGCTGA GCACCGCGTG CGTGTGTGA 2400  
GGTGATTGAG CGAGTCGGTC TCGTACTGG CTTCGGCCCG AGTGCCGTG TCCCGGCGCG 2460  
CGCGGTAAG AACAGTAGTA CTACCACCAG CTCTCCGTC CCCGGGGCCT TCAAATCGAG 2520  
CACGAGCCGG CTAGCTCCAG GCCCCCCAGT CCCGCAAGG GCGGGGGCC TTCCTGCTGG 2580  
15 TTCTAGCGGC ACGAGATCAC GGAGCCGGAT ACTGCTCTCG CGCGCCGAT TCGAGCTAGT 2640  
TCGTGCGCGC GGAGTCCTGC TGACGCGGGA TCCTGCCGAC GATCGACCCG CGCCGTCGCC 2700  
GAATTGGCG GCGGCTTCTT CGTGCCGTCT GGCATTACGT CGAGCGGGTG GTGGGCGTGC 2760  
GTGATTGGCC GGGTTTGGG TGCTTGCTGC TTCCGTCTCT GTGCTGAATG TCGGAATTCA 2820  
AGTCCCTTTT CCCCTTCGCT CCTGCTTGA GTGGACTAAC CTTAGTGTGG ACTTCAACAT 2880  
20

-230-

TTTTTTCATG TGATCTAGGG TCTTGCTGTT CTGTTTCTGC TGGCTGTTGA CTATCAGCTT 2940  
ACTGTTGCGG ATTGCGCACT TTCCCCCTGGC ACTGTTTCCG GAGGAATTTC CTGATTTTTT 3000  
TAGTTATTAG TGGTTAAATA GTACCATTAT GTCTTTGTTT GCTTTGTGCC ATTTTATAGCA 3060  
TCCAGTACAG AAAAAAAGGA ATAAACGTGC AAAACTGAAA AATAATAACC CGGTGCTGTT 3120  
TTCGCTAACC AGACAGAATT GATTCCACCA TTTTCCTGAT TTAGTTAGTA GTTAAATAGG 3180  
ACTACTATGT TTTTGTTC TGTTGTACCAT TTTAGCATCT AGTACAGAA AAGCGCACAC 3240  
ACATGCCAAA CCGAAAAGAA ATATCCCAAT GCTGCAATTC TACGCTAATC GGACATAAAT 3300  
GATTGATGCG CTAACAGACG GATTGTGTTCT TTGCTTTTC CCAGTGCTGA AGTTTGAGG 3360  
GGGCAATA

20 (2) INFORMATION FOR SEQ ID NO: 34:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 28 base pairs

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- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

5       (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

ATCGATCGGC CTCGGCTCCA ATTCATT

28

10      (2) INFORMATION FOR SEQ ID NO: 35:

      (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- 15       (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

      (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

20      GTTCCCAAAG GTCTCCAAGG

20

      (2) INFORMATION FOR SEQ ID NO: 36:

25       (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 37 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

30

      (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:

GCGGACTCGA GTCGACAAGC TTTTTTTTTT TTTTTT

37

35

      (2) INFORMATION FOR SEQ ID NO: 37:

- 232 -

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 34 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

5

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 37:

ACGCGTCGAC TAGTAGGTGC GGATGCTGCG CATG

34

10

## (2) INFORMATION FOR SEQ ID NO: 38:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

15

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 38:

GCGGACTCGA GTCGACAAGC

20

20

## (2) INFORMATION FOR SEQ ID NO: 39:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 29 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

30

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 39:

ACGCGTCGAC CATCCCATTTG TTGGCAACC

29

35

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## (2) INFORMATION FOR SEQ ID NO: 40:

## (i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 40:

10

GACTCATTGA GATCAAGTTC

20

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All of the compositions and methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the composition, methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.



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**CLAIMS:**

1. An isolated plant acetyl-CoA carboxylase enzyme.
- 5 2. The enzyme according to claim 1, wherein said enzyme is isolated from a dicotyledonous plant.
3. The enzyme according to claim 2, wherein said enzyme is isolated from soybean, rape, sunflower, tobacco, *Arabidopsis*, petunia, canola, pea, bean, tomato,  
10 potato, lettuce, spinach, carrot, alfalfa, or cotton.
4. The enzyme according to claim 3, wherein said enzyme is isolated from canola.
5. The enzyme according to claim 1, comprising the amino acid sequence of SEQ  
15 ID NO:20.
6. The enzyme according to claim 1, wherein said enzyme is isolated from a monocotyledonous plant.
- 20 7. The enzyme according to claim 6, wherein said enzyme is isolated from wheat, rice, maize, barley, rye, oats or timothy grass.
8. The enzyme according to claim 7, wherein said enzyme is isolated from wheat.
- 25 9. The enzyme according to claim 1 comprising the amino acid sequence of SEQ ID NO:10.
10. The enzyme according to claim 1 comprising a portion of a dicotyledonous acetyl-CoA carboxylase functionally linked to a portion of a monocotyledonous acetyl-  
30 CoA carboxylase.

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11. An isolated and purified plant acetyl-CoA carboxylase enzyme having the ability to catalyze the carboxylation of acetyl-CoA.
- 5 12. A purified DNA segment encoding plant or cyanobacterial acetyl-CoA carboxylase.
13. The DNA segment of claim 12, wherein said segment encodes canola acetyl-CoA carboxylase.
- 10 14. The DNA segment of claim 13, further defined as encoding the amino acid sequence of SEQ ID NO:20 or SEQ ID NO:31.
- 15 15. The DNA segment of claim 14, further defined as comprising SEQ ID NO:19 OR SEQ ID NO:30.
16. The DNA segment of claim 12, wherein said segment encodes wheat acetyl-CoA carboxylase.
- 20 17. The DNA segment of claim 16, further defined as encoding the amino acid sequence of SEQ ID NO:10 or SEQ ID NO:31.
18. The DNA segment of claim 17, further defined as SEQ ID NO:9 or SEQ ID NO:30.
- 25 19. The DNA segment of claim 12, defined further as a recombinant vector.
20. The DNA segment of claim 12, wherein said DNA is operatively linked to a promotor, said promotor expressing the DNA segment.
- 30

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21. The DNA segment of claim 12, wherein said DNA encodes a portion of a dicotyledonous acetyl-CoA carboxylase functionally linked to a portion of a monocotyledonous acetyl-CoA carboxylase.
- 5 22. A recombinant host cell comprising the DNA segment of claim 12.
23. The recombinant host cell of claim 22, defined further as being a prokaryotic cell.
- 10 24. The recombinant host cell of claim 23, further defined as a bacterial or cyanobacterial host cell.
25. The recombinant host cell of claim 22, defined further as being a eukaryotic cell.
- 15 26. The recombinant host cell of claim 25, further defined as a yeast cell or a plant host cell.
27. The recombinant host cell of claim 26, wherein said cell is a monocotyledonous plant cell.
- 20 28. The recombinant host cell of claim 24, wherein the bacterial host cell is *E. coli*.
29. The recombinant host cell of claim 24, wherein the cyanobacterial host cell is *Synechococcus* or *Anabaena*.
- 25 30. The recombinant host cell of claim 22, wherein the DNA segment is introduced into the cell by means of a recombinant vector.
31. The recombinant host cell of claim 22, wherein the host cell expresses the DNA  
30 segment to produce the encoded acetyl-CoA carboxylase protein or peptide.

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32. The recombinant host cell of claim 22, wherein the expressed acetyl-CoA carboxylase protein or peptide includes a contiguous amino acid sequence from SEQ ID NO:2; SEQ ID NO:4; SEQ ID NO:6; SEQ ID NO:8; SEQ ID NO:10; SEQ ID NO:12; SEQ ID NO:20 or SEQ ID NO:31.

33. A method of using a DNA segment that encodes an isolated acetyl-CoA carboxylase, comprising the steps of:

- 10 (a) preparing a recombinant vector in which an acetyl-CoA carboxylase-encoding DNA segment is positioned under the control of a promoter;
- (b) introducing said recombinant vector into a recombinant host cell;
- 15 (c) culturing the recombinant host cell under conditions effective to allow expression of an encoded acetyl-CoA carboxylase protein or peptide; and
- (d) collecting said expressed acetyl-CoA carboxylase protein or peptide.

20

34. An isolated nucleic acid segment characterized as:

- 25 (a) a nucleic acid segment comprising a sequence region that consists of at least 14 contiguous nucleotides that have the same sequence as, or are complementary to, 14 contiguous nucleotides of SEQ ID NO:1; SEQ ID NO:3; SEQ ID NO:5; SEQ ID NO:7; SEQ ID NO:9; SEQ ID NO:11; SEQ ID NO:19 or SEQ ID NO:30; or

30

- (b) a nucleic acid segment of from 14 to about 10,000 nucleotides in length that hybridizes to the nucleic acid segment of SEQ ID NO:1; SEQ ID NO:3;

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SEQ ID NO:5; SEQ ID NO:7; SEQ ID NO:9; SEQ ID NO:11; SEQ ID NO:19 or SEQ ID NO:30; or the complements thereof, under standard hybridization conditions.

5     35.     The nucleic acid segment of claim 34, further defined as comprising a sequence region that consists of at least 14 contiguous nucleotides that have the same sequence as, or are complementary to, 14 contiguous nucleotides of SEQ ID NO:1; SEQ ID NO:3; SEQ ID NO:5; SEQ ID NO:7; SEQ ID NO:9; SEQ ID NO:11; SEQ ID NO:19; or SEQ ID NO:30.

10

36.     The nucleic acid segment of claim 34, further defined as comprising a nucleic acid segment of from 14 to about 10,000 nucleotides in length that hybridizes to the nucleic acid segment of SEQ ID NO:1; SEQ ID NO:3; SEQ ID NO:5; SEQ ID NO:7; SEQ ID NO:9; SEQ ID NO:11; SEQ ID NO:19; or SEQ ID NO:30, or the complements thereof, under standard hybridization conditions.

15

37.     The nucleic acid segment of claim 34, wherein the segment comprises a sequence region of at least 14 contiguous nucleotides from SEQ ID NO:19 or SEQ ID NO:30, or the complement thereof, or wherein the nucleic acid segment hybridizes to the nucleic acid segment of SEQ ID NO:19 or SEQ ID NO:30, or the complement thereof, under standard hybridization conditions.

20

38.     The nucleic acid segment of claim 34, wherein the segment comprises a sequence region of at least 14 contiguous nucleotides from SEQ ID NO:9 or SEQ ID NO:11, or the complement thereof, or wherein the nucleic acid segment hybridizes to the nucleic acid segment of SEQ ID NO:9 or SEQ ID NO:11, or the complement thereof, under standard hybridization conditions.

25

39.     The nucleic acid segment of claim 34, wherein the segment comprises a sequence region of at least 14 contiguous nucleotides from SEQ ID NO:7, or the

30

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complement thereof, or wherein the nucleic acid segment hybridizes to the nucleic acid segment of SEQ ID NO:7, or the complement thereof, under standard hybridization conditions.

5     40.     The nucleic acid segment of claim 34, wherein the segment comprises a sequence region of at least 14 contiguous nucleotides from SEQ ID NO:5, or the complement thereof, or wherein the nucleic acid segment hybridizes to the nucleic acid segment of SEQ ID NO:5, or the complement thereof, under standard hybridization conditions.

10

41.     The nucleic acid segment of claim 34, wherein the segment comprises a sequence region of at least 14 contiguous nucleotides from SEQ ID NO:1 or SEQ ID NO:3, or the complement thereof, or wherein the nucleic acid segment hybridizes to the nucleic acid segment of SEQ ID NO:1 or SEQ ID NO:3, or the complement thereof,  
15     under standard hybridization conditions.

42.     The nucleic acid segment of claim 34, wherein the segment comprises a sequence region of at least about 20 nucleotides; or wherein the segment is about 20 nucleotides in length.

20

43.     The nucleic acid segment of claim 34, wherein the segment comprises a sequence region of at least about 30 nucleotides; or wherein the segment is about 30 nucleotides in length.

25

44.     The nucleic acid segment of claim 34, wherein the segment comprises a sequence region of at least about 50 nucleotides; or wherein the segment is about 50 nucleotides in length.

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45. The nucleic acid segment of claim 34, wherein the segment comprises a sequence region of at least about 100 nucleotides; or wherein the segment is about 100 nucleotides in length.
- 5 46. The nucleic acid segment of claim 34, wherein the segment comprises a sequence region of at least about 200 nucleotides; or wherein the segment is about 200 nucleotides in length.
- 10 47. The nucleic acid segment of claim 34, wherein the segment comprises a sequence region of at least about 500 nucleotides; or wherein the segment is about 500 nucleotides in length.
- 15 48. The nucleic acid segment of claim 34, wherein the segment comprises a sequence region of at least about 1000 nucleotides; or wherein the segment is about 1000 nucleotides in length.
49. The nucleic acid segment of claim 34, wherein the segment comprises a sequence region of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, or SEQ ID NO:7.
- 20 50. The nucleic acid segment of claim 34, wherein the segment comprises a sequence region of SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:19 or SEQ ID NO:30.
- 25 51. The nucleic acid segment of claim 34, wherein the segment is up to 10,000 basepairs in length.
52. The nucleic acid segment of claim 34, wherein the segment is up to 5,000 basepairs in length.
- 30 53. The nucleic acid segment of claim 34, wherein the segment is up to 3,000 basepairs in length.

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54. The nucleic acid segment of claim 34, wherein the segment is up to 1,000 basepairs in length.

5 55. A method for detecting a nucleic acid sequence encoding a plant acetyl-CoA carboxylase, comprising the steps of:

- (a) obtaining sample nucleic acids suspected of encoding a plant acetyl-CoA carboxylase;
- 10 (b) contacting said sample nucleic acids with an isolated nucleic acid segment encoding acetyl-CoA carboxylase under conditions effective to allow hybridization of substantially complementary nucleic acids; and
- 15 (c) detecting the hybridized complementary nucleic acids thus formed.

56. The method of claim 55, wherein the sample nucleic acids contacted are located within a cell.

20 57. The method of claim 55, wherein the sample nucleic acids are separated from a cell prior to contact.

58. The method of claim 55, wherein the isolated plant acetyl-CoA carboxylase-encoding nucleic acid segment comprises a detectable label and the hybridized  
25 complementary nucleic acids are detected by detecting said label.

59. A nucleic acid detection kit comprising, in suitable container means, an isolated plant or cyanobacterial acetyl-CoA carboxylase-encoding nucleic acid segment and a detection reagent.

30



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60. The nucleic acid detection kit of claim 59, wherein the detection reagent is a detectable label that is linked to said acetyl-CoA carboxylase nucleic acid segment.
61. An enzyme composition, free from total cells, comprising a purified acetyl-CoA carboxylase that includes a contiguous amino acid sequence from SEQ ID NO:2; SEQ ID NO:4; SEQ ID NO:6; SEQ ID NO:8; SEQ ID NO:10; SEQ ID NO:12; SEQ ID NO:20; or SEQ ID NO:31.
62. The composition of claim 61, comprising a peptide that includes a 15 to about 50 amino acid long sequence from SEQ ID NO:2; SEQ ID NO:4; SEQ ID NO:6; SEQ ID NO:8; SEQ ID NO:10; SEQ ID NO:12; SEQ ID NO:20; or SEQ ID NO:31.
63. The composition of claim 61, comprising a peptide that includes a 15 to about 150 amino acid long sequence from SEQ ID NO:2; SEQ ID NO:4; SEQ ID NO:6; SEQ ID NO:8; SEQ ID NO:10; SEQ ID NO:12; SEQ ID NO:20 or SEQ ID NO:31.
64. The composition of claim 61, wherein the protein or peptide is a recombinant protein or peptide.
65. A purified antibody that binds to a plant or cyanobacterial acetyl-CoA carboxylase protein or peptide.
66. The antibody of claim 65, wherein the antibody is a monoclonal antibody.
67. A method for detecting an acetyl-CoA carboxylase peptide in a biological sample, comprising the steps of:
- (a) obtaining a biological sample suspected of containing an acetyl-CoA carboxylase peptide;

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- (b) contacting said sample with a first antibody that binds to a plant acetyl-CoA carboxylase protein or peptide, under conditions effective to allow the formation of immune complexes; and
- 5 (c) detecting the immune complexes so formed.

68. The method of claim 67, wherein said first antibody is linked to a detectable label and the immune complexes are detected by detecting the presence of the label.

10 69. The method of claim 67, wherein said immune complexes are detected by means of a second antibody linked to a detectable label, the second antibody having binding affinity for said first protein, peptide or antibody.

70. An immunodetection kit comprising, in suitable container means, a first  
15 antibody that binds to an acetyl-CoA carboxylase protein or peptide, and an immunodetection reagent.

71. A process for determining resistance to herbicides of the  
aryloxyphenoxypropionate or cyclohexanedione class in a plant, comprising:

20

- (a) obtaining a sample from said plant; and
- (b) testing for the presence of an acetyl-CoA carboxylase enzyme capable of conferring resistance to said plant in said sample.

25

72. The process according to claim 71, wherein the presence of an acetyl-CoA carboxylase enzyme conferring said resistance is determined by identifying the presence of an acetyl-CoA carboxylase polypeptide in said plant.

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73. The process according to claim 71, wherein the presence of an acetyl-CoA carboxylase enzyme conferring said resistance is determined by identifying the presence of an acetyl-CoA carboxylase-encoding nucleic acid segment in said plant.

5 74. The process according to claim 71, wherein said sample is obtained from a progeny plant of a parent plant that includes a herbicide-resistant acetyl-CoA carboxylase transgene.

10 75. The process according to claim 71, wherein said sample is suspected of containing a fusion protein comprising a portion of a dicotyledonous plant acetyl-CoA carboxylase functionally linked to a portion of a monocotyledonous plant acetyl-CoA carboxylase or one or more domains of a cyanobacterial acetyl-CoA carboxylase.

15 76. A process for identifying herbicide resistant variants of a plant acetyl-CoA carboxylase enzyme, comprising the steps of:

- 20 (a) transforming a cyanobacterium or a yeast cell with a candidate DNA molecule that encodes an engineered plant acetyl-CoA carboxylase enzyme suspected of conferring herbicide resistance to form a transformed cyanobacterium;
- (b) inactivating cyanobacterial or yeast acetyl-CoA carboxylase;
- (c) exposing said transformed cyanobacterium or said transformed yeast cell  
25 to a herbicide that inhibits acetyl-CoA carboxylase activity;
- (d) identifying transformed cyanobacteria or transformed yeast cells that are resistant to said herbicide; and

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- (e) characterizing DNA that encodes acetyl-CoA carboxylase from the cyanobacteria or yeast cells of step (d).

77. The process of claim 76, wherein said acetyl-CoA carboxylase enzyme is a fusion protein comprising a portion of a dicotyledonous plant acetyl-CoA carboxylase functionally linked to a portion of a monocotyledonous plant acetyl-CoA carboxylase or one or more domains of a cyanobacterial acetyl-CoA carboxylase.

78. The process of claim 76, wherein said acetyl-CoA carboxylase enzyme is an engineered dicotyledonous plant acetyl-CoA carboxylase, or a portion of an engineered dicotyledonous plant acetyl-CoA carboxylase functionally linked to a portion of a monocotyledonous plant acetyl-CoA carboxylase or one or more domains of a cyanobacterial acetyl-CoA carboxylase, or an engineered cyanobacterial acetyl-CoA carboxylase enzyme.

79. A process of modifying the oil content of a plant cell, comprising expressing in a plant cell a DNA segment that encodes a plant or cyanobacterial acetyl-CoA carboxylase or the complement of said DNA segment.

80. The process according to claim 79, comprising incorporating into said plant cell a DNA segment that encodes a plant or cyanobacterial acetyl-CoA carboxylase polypeptide, wherein said cell expresses the acetyl-CoA carboxylase enzyme.

81. The process according to claim 80, wherein said plant cell is a monocotyledonous plant cell.

82. A process of increasing the herbicide resistance of a monocotyledonous plant, comprising incorporating into said plant a transgene comprising a DNA segment encoding a plant or cyanobacterial acetyl-CoA carboxylase polypeptide resistant to herbicide inactivation, the plant expressing the polypeptide.

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83. The process according to claim 82, wherein said acetyl-CoA carboxylase polypeptide is a dicotyledonous plant acetyl-CoA carboxylase polypeptide.

5 84. The process according to claim 81, wherein said plant acetyl-CoA carboxylase polypeptide comprises the amino acid sequence of SEQ ID NO:10; SEQ ID NO:20 or SEQ ID NO:31.

85. The process according to claim 81, wherein said plant acetyl-CoA carboxylase  
10 polypeptide is encoded by the DNA sequence comprising SEQ ID NO:9; SEQ ID NO:19, or SEQ ID NO:30.

86. The process according to claim 81, wherein said cyanobacterial acetyl-CoA  
carboxylase polypeptide comprises the amino acid sequence of SEQ ID NO:2; SEQ ID  
15 NO:4; SEQ ID NO:6; SEQ ID NO:8; or SEQ ID NO:12.

87. The process according to claim 81, wherein said cyanobacterial acetyl-CoA  
carboxylase polypeptide is encoded by the DNA sequence comprising SEQ ID NO:1;  
SEQ ID NO:3; SEQ ID NO:5; SEQ ID NO:7; SEQ ID NO:11; or SEQ ID NO:30.  
20

88. A transgenic plant having incorporated into its genome a transgene that encodes  
a plant or cyanobacterial acetyl-CoA carboxylase.

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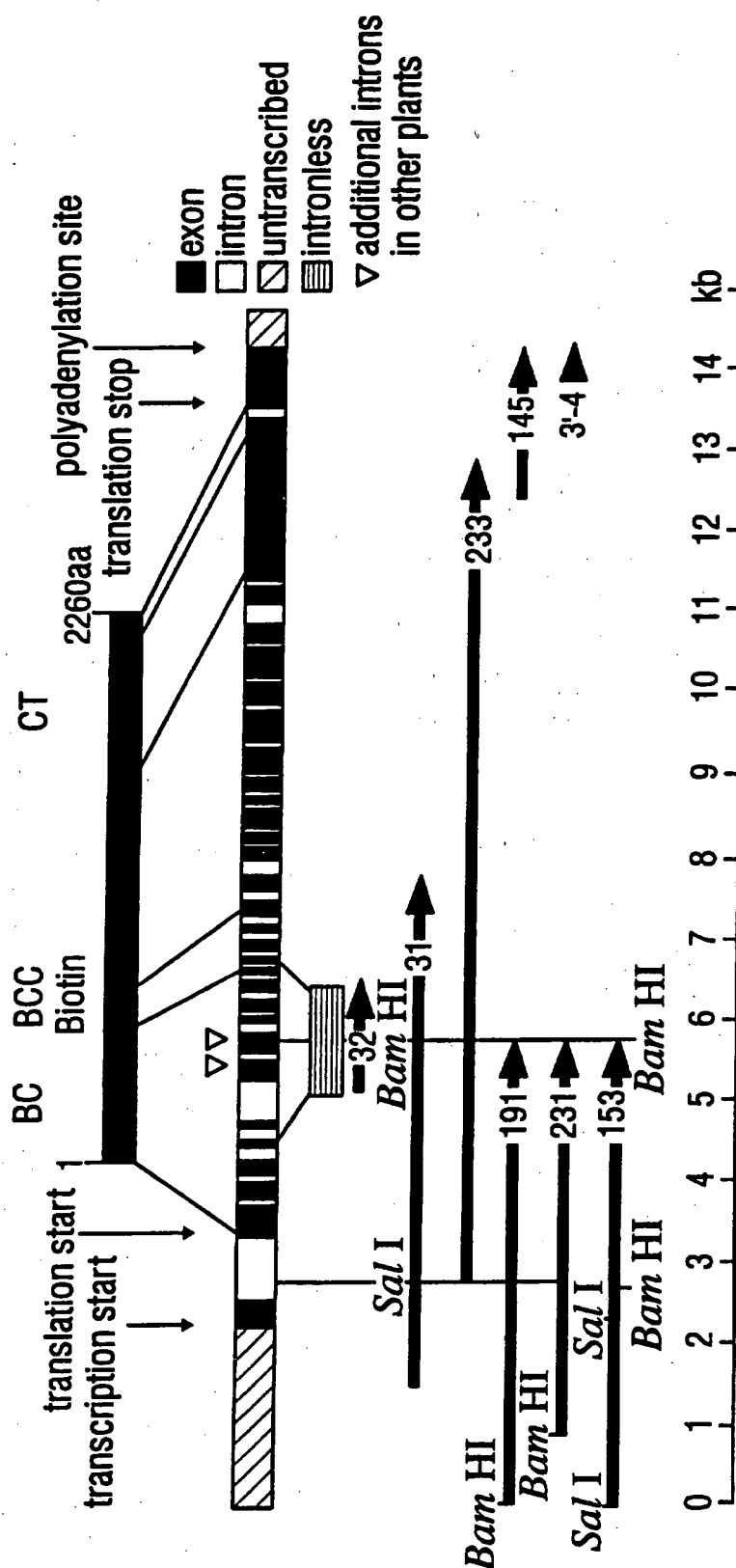


FIG. 1

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3'-2 GAGCTACAGGAGGGAGGTTTTTCA-----GAAACTGAACACATTGTTTGCACATAGGTAGGAGCCATCTCATCTCAGGACAAATTGTATGTTTATTGTTATTATTA  
3'-1 GAGCTACAGGAGGGAGGTTTTTCA-----ACTGAACACATTGTTTGCACATAGGTAGGAGCCATCTCATCTCAGGACAAATTGTATGTTTATTGTTATTATTA  
3'-4 GAGCTACAGGAGGGAGGAGTTTTTCAGCGGAA-----ACTGAACACATTGTTTGCACATAGGTAGGAGCCATCTCATCTCAGGACAAATTGTATGTTTATTGTTATTATTA  
3'-10 GAGCTACAGGAGGGAGGAGTTTTTCAGCGGAAAGAAACTGAACACATTGTTTGCACATAGGTAGGAGCCATCTCATCTCAGGACAAATTGTATGTTTATTGTTATTATTA  
\*\*\*\*\*  
GATAGGTACACACAAAGCATATGATCTCTGGATAGATATTCTGGTGTGAGTTGTGCPATGCAAGATTTCATCTTAATTTA (A) n  
GATAGGTACACACAAAGCATATGATCTCTGGATAGATATTCTGGTGTGAGTTGTGCPATGCAAGATTTCATCTTAATTACGAGATACG-TGTGAT-----GGTGGATGTGATAGTCTT  
GATAGGTACACACAAAGCATATGATCTCTGGATAGATATTCTGGTGTGAGTTGTGCPATGCAAGATTTCATCTTAATTACGAGATACGATGTGATGATCGGTGGATGTGTTGTTGT  
GATAGGTACACACAAAGCATATGATCTCTGGATAGATATTCTGGTGTGAGTTGTGCPATGCAAGATTTCATCTTAATTACG (A) n  
\*\*\*\*\*

FIG. 2-1

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AGTTTCCTGGTGGGAGGAACCGTGAAGTTTCCTTTCTGCTGAGTTATGATGATATA--CCCTGAGAACCTTGGGGTGATATGATGATGTTT-ATCAGTTTCATGATATAATGAAATT  
 AGTTTCCTCAGTGGCAGGAGATCCGAGTTTCCTTACCGCTGAGTTATGATGATGATGATTAACCCCTGAGAACCTTGGGG-TGATATGATGAGACGTTTATCAGTTTCATGAGAAATGAAATT  
 \*\*\*\*\* \*\*\*\* \* \* \* \* \* \*\*\*\*\* \*\*\*\* \* \* \* \* \*

GG-----TATGGATGTTTCTTCCTC (A) n  
 GGAGCCGAGGCCCTTACATCAGTTTCTTCT (A) n  
 \*\* \* \* \* \*

FIG. 2-2



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1 gccgcgcca ccaggggccat gcggcccaac taccgctcgt cccggtctag accacgcccc  
 61 ccacctgccc cgccccaccc ccccccaac tcctccatga atgcacgcat ttcacgctc  
 121 caaccacaac gcagcagccc cagcaccagc ggccctggcg acgcgcgcg cattatacc  
 181 acgcaattcc atctggatct ccacctggcc gcagcacggg ttctctctc cctccccgcg  
 241 cggcattccg tcgaacggct ttgcggcgcg cctccggacg gacccacggt aagctccccc  
 301 tgcccttgct atgccccctg ttctgcacgc atctccgat ttccgtgga gcgctccgccc  
 361 tccgctatg cgtgcggcg attgactggc cgggacttgc catggactcg tactgaccag  
 421 tgatgtactc gctcgtagc ctctccgcc acgcccgcct caaatcgagc gcgctaggc  
 481 tgccctcagg ccccaatcca agcagcgag cgcagggcct tcctgctgat tctctctcag  
 541 cgccaggaga tcacgggacc agataccact gctagcagtc gacccgtgcc gtcgcccgat  
 601 tgccgggttc gccccgtctg gcattacgtc gacgggtgg tggcgcgcg cgaactggccc  
 661 ggttttgggc acactgttg cttaactcct tctgctgaat gccggaatc aagtcattt  
 721 cctcttttgc tctgcttgg actaacagc cccctagtgt ggaactacagc atttttttcg  
 781 cgtattttta atgtgatctc tggcttctgct ctctctggtc tgctggttgc tgactagaat  
 841 tctgcaactc cccatggcac tcttgccgga ggaatttccc gathtagta gccgttaatt  
 901 agtgccacca tgtgtgtgt ttctgtagta ccatatttagc atctggtaca gaaaaagggc  
 961 acacacatgc caaacgaaa agaaatatcc cagtgtctga attctacgt aatcgggacat  
 1021 aatgattga tgcgctaacy gacggacttg ttcttttgc tttccacgc ctgaaagggtg  
 1081 gagggggcaa taatggtgga atctgaccaa ataaacggga cggccacacag gatgtcctcg  
 1141 gtcgatgaat tctgtaagc gctcgggggt gactcgccga tacacagcgt gctggttgcc  
 1201 aacaatggga tggctgcggt caaattcatg cgcagcatcc gcacctggc cttggagacc  
 1261 ttggggaacg agaaggccat tctcttgggt gctatggcaa ctccagagga cctcaggata  
 1321 aatgcggagc acataagaat cgcgcaccag ttcttagaag ttccctggtgg aacgaacaat  
 1381 aacaactatg caaatgtaca gctcatagtg gaggttagtg cagttgatca tcttttttca  
 1441 cctactactt atggattacc atgttcatta tgcctggatac ttgactagtt ataatcttt  
 1501 ctgattcacc tgtcctgtca cagatagcag agagaactcg ggtttctgca gtttggcctg  
 1561 gctggggtca tgcttctgag aaccagaac ttccagacgc gctcatgga aagggaatca  
 1621 tttttcttgg gccaccatca gccgcgatgg gggcactagg cgataagatt ggttcttctc  
 1681 ttattgcaca agcagcagga gttccaactc ttccatggag cgggtcacat gtatgtatac  
 1741 cttgtcctat ttcttttatgg ttttgcctct atgtttttct cttccaccat gtgtatttct  
 1801 caaaactaaa tcaatacacg ctgtagggtga agtttccgca agaaacctgc cactcaatc  
 1861 ctgaggagat ctataagaac gcttgtgtgtt caactacaga cgaagcagtt gctagtgtc

FIG. 3-1

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1921 aggtggtggg gtatcctgca atgatcaagg catcatgggg cgggggtggg aaggaataa  
 1981 ggaagggtgg tattcttttc atcttttcaa tcatctctca ccttagttat atggaatgct  
 2041 ctactagaaa caattacatg taatttccac tgttcatttg aaatgaagtc caagttttct  
 2101 gcaattattg tatattaacc aaagatgttt tttatgtcat caaatgggtt tataggtaca  
 2161 taatgatgat gaggtcagag cattgtttaa gcaagtgcga ggagaagtcc cggatcgcc  
 2221 tataattatt atgaaggtag catctcaggt gatacgtgat aagctgataa cagccattat  
 2281 tttctgttgt atcttttgtt tactcatgtt cagtatttcag cgagtgcctc tctgtactg  
 2341 atatagttca tttagctaaa atcttgctt tctgtacttt ctttgtagag ccgacatcta  
 2401 gaggttcagt tgctctgtga caagcatggc aacgtggcag cactgcacag tcgagactgt  
 2461 agtgttcaaa gaaggcacca aaaggttagt tattctctg aagcattggg ttgttcaata  
 2521 tcagttttgt tggaattagt cttagccaaa catttgtga ttgagtactg gtagaagttc  
 2581 tacagcttca ggggaataaa aacttcattg gacaatgtag caatcatata gtactgttta  
 2641 gcaagtgca aaatgttgca ggagctatag caaatttatg tcgtggcatt tctttaaag  
 2701 gaatcattta ttactgttag ttatacttat actgtactaa atagttgaat gttgcatttt  
 2761 gaattcaaga acaactttt tcttctata gtgatatatg tgttgtactt gaagtttttg  
 2821 aactcagaat attgaaaagt ctagtactg tattacagat tattttgtaa ccaaaaaaat  
 2881 ttaactagtg caagacagat aatagcagag aagtcttagc aaaattatat ttattttact  
 2941 tctcacgata tatatacttg tgaacacagat cattgaggag ggaccaatta cagttgctcc  
 3001 tccagaaaca attaaagagc ttgagcaggc agcaaggcgg cttgctaaa gtgtgcaata  
 3061 tcagggtgct gctacagtag aatatctgta cagcatggaa acaggcgaat actatttctt  
 3121 ggagcttaat ccaagggttc aggtagaaca ccctgtgacc gaatggattg ctgaaaataaa  
 3181 cttacctgca tctcaagttg tagtaggaat gggcatacca cttacaaca ttccaggtag  
 3241 gccagttgtc caacttgatg gttgatgata ttatctcttt ccccccac taatcaatat  
 3301 aaggataaact gcagagatca gacgttttta ttggaatagaa catggagggtg gctatcacgc  
 3361 ttggaaggaa atatcagctg ttgcaactaa atttgatctg gacaaagcac agtctgtaaa  
 3421 gccaaagggt cattgtgtag cagttagagt tactagcgag gatccagatg atgggtttaa  
 3481 gcctacaagt ggaagagtgg aagagctgaa ctttaaaagt aaaccaatg ttggggccta  
 3541 tttctctgtt aaggcaagtt tgcattccatg cagaatgac ttgtatacca catgacatgt  
 3601 cacaacagct gcagcttatac attacccttg agttttcctg tttcttatgt cgataaaattt  
 3661 cctgggttaa aactgtatct tgtgtggcaa acctaacctg aatcatcgtt ttttgtttca  
 3721 gtccggaggt gcaattcatg agttctctga tccccagttt ggtaagtgat gtgcgtaaat  
 3781 ttctgtttcc tcatatatct catgatgatg cttctcttaa acagcatgcc ttttttcgca

FIG. 3-2

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3841 ggtcatgttt ttgcttttgg ggaatctagg tcattggcaa tagccaatat ggtacttggg  
 3901 ttaaaagaga tccaaattcg tggagagata cgcactaatg ttgactacac tgtggatctc  
 3961 ttgaatgtaa gataacccca cagtaaacat gttctctgat tacatgggtac atttattaag  
 4021 aaaaacatgg tacaattttg tgtgtgtaat ttatgttcaa aatttttcat atctccaggc  
 4081 tgcagagtac cgagaaaaata catggtcacac ttgttggcta gacacagaa tagcaatgcg  
 4141 tgttagagca gagaggcccc agattttatt aggttggtt ttctggagctc tataatgtg  
 4201 atttcttttt ctgggggaact atgattttatt atttcaggaa gacttttcat acaagatcca  
 4261 ttttccatcc tcaaatactg tgtttcttat atttcaggaa gcaccaagca ggagctcgag  
 4321 tgttgaacc gattatgttg gttatctcag taaagggtcaa ataccaccaa aggtacatac  
 4381 tataatgaga atgttcttac tgtttatat ccaatttcta tatgaataaa actgtctaac  
 4441 tctttccgtt cacagcacat ctctcttctc aatttgactg taacactgaa tatagatggg  
 4501 agcaaatata cggtaattat ctataatttt ctctttaatc ttatccatgc cataccatc  
 4561 taatccagtt ggtatccttg tcacatctgc taattattat tttcttctgc agattgagac  
 4621 agtacgaggt ggaccccgta gctacaaatt aagaattaat gaatcagagg ttgaagcaga  
 4681 gatacatctg ctgcgagatg gcggactctt aatgcaggta gatataatcta ccaagttttt  
 4741 atacaagcgc aatctatcta atttctttt tatttgaaa tggctgacc aattttcaat  
 4801 tgtgaatttt ctagtggat ggaacagtc atgtaattta cgccgagaca gaagctgctg  
 4861 gcacgcgtct tctaataaat gggagaacat gcttattaca ggtgaagata gctagatctg  
 4921 tactctcttc ttggttccta tgtaatatag ggtttgtttc agttgtaact ctagtgcga  
 4981 attgtatgaa aatacataaa ttaattatgt cctctgaatg atatattaca gaaagagcat  
 5041 gacccctcca ggttgttggc tgataacca tgcaagcttc ttcggttttt ggtcgcggat  
 5101 ggttctcatg tggttgctga tacgccatat gctgaggtgg aggtgatgaa aatgtgcatg  
 5161 ccactgttac taccggcctc tgggtctcatt cactttgtca tgcctgaggg tcaggccatg  
 5221 caggttcctc cccctcctct gtttgcagca ctagatgtac attctgacaa aagtactata  
 5281 tggttcatgc tcgtaataata cgtgcatcct ttaaatagta gctgaaatgg ctgtctttgt  
 5341 gcaggcgagt gatctgatag caaggttgga tcttgatgac ccatcttctg tgagaagggc  
 5401 tgaaccattt catggcacct tccaaaact tggacctcct actgctattt ctggcaaaagt  
 5461 tcacaaaaag ttgtgctgcaa gttgaaattc tgccacatg atccttgcat gatataaaca  
 5521 taacatcaat catgtaaggc acatcaaaact gtcagtgtat acttgttctt ccaactttct  
 5581 tttcccttgt ctatcacatt gccatgggaa aacagagcat gatttcttct acagagagaa  
 5641 actaacctct taattgtgac aaactatacc atctttcttc aatcaataag ttctgactg  
 5701 taccttttct ttcagggttg acaagatttg ctgaactgcc tagacagccc tgagctccct  
 5761 ttctgcagt ggcaagaact catgtccgtt ttggcaaccc gactcccgaa agatcttagg

FIG. 3-3

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5821 aatgaggatga ataagtattc aagttatatt tttttatctt agagttatta ttccattttt  
 5881 catttcggct gcataatcaaa tggataactg atttacctgt tctcagttgg atgctaagta  
 5941 caaggagtat gagttgaatg ctgacttccg gaagagcaag gattccctg ccaagttgct  
 6001 aaggggagtc attgaggtca gtttgagact gttacttggc atcccttcc tttttatgtg  
 6061 tcatgttgtt tccttacaaa gtcatcatg caggctaact ttgcatactg ttccgagaaa  
 6121 gatagggtca ctagtggag gcttgtagag ccacttatga gtctgggtcaa gtcatatgag  
 6181 ggtggaagag aaagccatgc tcgtgctggt gtcaagtctc tgtttgagga gtatttatct  
 6241 gttgaagaac tcttcagcga tgacattcag gtaactattt ataattgctt ggaatggttt  
 6301 gatcgatgct cactttctga ccaaaacgtg cttaaacatg caaaagacct tgagaaggctc  
 6361 tctcagtcgt atgtgataga acgtctacga cttcaacatg tgcactctgt tgacttgata  
 6421 tatatatattg tgttctccca ccaggtaatg tcttctattg tgcaatctgt tgacttgata  
 6481 tgcaaaaattt tcgtgctgac aatttctgtt cttttgaagg gtgtgaaaaa taaaaataaa  
 6541 ttaatactac ggcttatgga agcattggtc tatccaaatc gcataactcg ggttaaaatt gagtttggat  
 6601 ttgattcgtc tctctgcctt gaaccataca gcatactcgg ggttaaaatt gagtttggat  
 6661 gatctgcac tatattattt gcacattgat atgatatgct agaaaaataa aataaatcta  
 6721 ttgtaattga tgcagctggc gcttaaaaga agccaaactc ttgagcacac caaattgagt  
 6781 gaactccgca caagcatagc aagaagcctt tcagagctgg agatgtttac tgaggaagga  
 6841 gagcggattt caacacctag gaggaagatg gctatcaatg aaaggatgga agattttagta  
 6901 tgtgcaccgg ttgcagttga agacgcctt gtggctttgt ttgatacacag tgatcctact  
 6961 cttcagcggg gagtagtcga gacatacata cgcagattgt atcaggtatc actgattttt  
 7021 tttttacta cactctttct tgagacaaat agaacattaa caaatttatg ccggctaact  
 7081 cacaatcacc ttccagcatt atcttgcaag gggcagcgtc cggatgcaat ggcataggctc  
 7141 tgggtctaatt gctttatggg aattctctga agagcatatt gaacaaagaa atgggcaatc  
 7201 tgcgtcactt cttaaagccac aagtagagga tccaattggc agcgatggg gtgtaatgg  
 7261 tgtaatcaag tctcttcagc ttctgtcaac tgcaattgaa gctgcattaa aggagacttc  
 7321 acactacgga gcaggtgttg gaagtgtctc aaatggtaat cctataaaat tgaacggcag  
 7381 caatatgctg cacattgctc tggttggtat caacaatcag atgagcactc ttcaagacag  
 7441 gtttgtttac actctattct tatgtgtttt gttgttattg cacaggagac gagtgtgatt  
 7501 ctgtgaactg gtcgttaatt tcatgatttt ttagtacct ctccactct gttttctctt  
 7561 tatagtgggtg atgaggatca agcgcaagaa aggatcaaca aactctcaa gattttgaag  
 7621 gataacacta taacatcaca tctcaatggt gctggtgta ggttgtcag ctgcattatc  
 7681 caaagagatg aaggcggttc accaatgcgc cactccttca aatggtcatc tgacaagta  
 7741 tattatgagg aggaccgat gctccgcat gtggaatctc ctttgtccac cttccttgaa

FIG. 3-4

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7801 ttggtattca gcttttgttt tggcttatgt tcccttcaat aataccagta aataccagta cctcttaaca  
 7861 gtttatgtgt aaatacagga caaagtgaat ttagaaggtt acaatgacgc gaaatacacc  
 7921 ccatcacgtg atcgccagtg gcacatgtac acactagtaa agaacaagaa agatccgaga  
 7981 tcaaatgacc aaaggatgtt tcttcgtacc atagtcagac agccaagtgt gaccaatggg  
 8041 tttttgtttg gaagtattga taatgaagt caagcctcgt caatcattcac atctaacagc  
 8101 atactcagat cattgatggc agctctagaa gaaatagagt tgcgtgctca cagtgaagact  
 8161 gggatgtcag gccactccca catgtatctg tgcataatga gagaacaacg gttgtttgat  
 8221 ctaattccat cttcaaggtc agtcaaaatt tatttatgtt ctcaacagat tataattgcat  
 8281 taatatgtt catagatgtt cacttggttt ttgcttctca ttatgttagg atgacgaatg  
 8341 aagttggtca agatgagaag acagcatgca cactattgaa gcataatggt atgaatatat  
 8401 atgagcatgt tgggtcagg atgcatcgcc tttccgtgtg ccagtgggaa gtgaagctat  
 8461 ggttggattg tgatggcag gctaatggtg cttggagagt tgtgttacc agtgaactg  
 8521 gcaataacctg cactgttgat gtaagttacc tttagctattg cactgctacg cgagcattat  
 8581 catctacagt ttgcaataa ctacctctga tggataaagc cccacagatc atcaaatatg  
 8641 atttgttag cttatctagt tagtgaatag aaaatgttca tcacccccat tatgagtga  
 8701 atgggtaac tctcaatttt tgcctttaa agttctatta aacactactt aaaagacttg  
 8761 taagtaccag gtaccatttt ctctttattg ctcttatgct tgaattattt tgactttcag  
 8821 atttaccag aagtggagg ccccaataca cataagcttt tctatcgctc tgccacaccc  
 8881 acagctggtc ctttgcattg cattgcattg gaaaccacat actgctatga tttcccatg  
 8941 gacctgaac gtgctgctgc taggaaaaat ccatgagccat acaaaccttt ggatgctatt  
 9001 gtgcgttagc tacatctctt ttcttttttt ctctacaatt ggtaacatg attaaactaag  
 9061 attggttaata atactctgtc cgcaggcatt tgaacacagca ttgaagaagt catgggaatc  
 9121 tggattttca catgttgca aatctaata gcataaaccag cggatgctg aagtgaacaga  
 9181 gcttatattt gctgattcaa ctggatcatg ggtactcct ttggttccag ttgagcgtcc  
 9241 tccaggtagc aacaattttg gtgtgtgtgc ttggaacatg aagctctcca caccagaatt  
 9301 tccagggtggc cgggagatta tagttgtgc aatgatgtg acatttaaag ctgggtcttt  
 9361 tggtcctaga gaagatgcat tctttgatgc tgtcacaat cttgcttctg agaggaaaat  
 9421 tctctaatc tacttgtcag caactgctgg tgcaaggctc ggtgtagcag aggaaataaa  
 9481 ggcattgcttc catgttggat ggtctgatga ccagagccct gaactggtt ttcactacat  
 9541 ttacctcact gaacaagatt attcagctt aagctcttca gttatagccc atgagctaaa  
 9601 agtaccagaa agcggagaaa ccagatgggt tgttgatacc atgttgga aagaggacgg  
 9661 acttgggtgt gagaatctac atggaagtgg tggcattgcc atgctact ctaaggcata

FIG. 3-5

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9721 tagagagaca ttactctga catttgtgac tggccgagct attggaattg gggcctatct  
 9781 tgctcgggta ggaatgcggt gtatacaacg tcttgatcaa ccaattattt tgactgggta  
 9841 ttctgcactg aacaagctcc tggggcgga atggagtgt tcatctcact gtgcagatgc aactgggtgg  
 9901 ccccaaatc atggctaca atggagtgt tcatctcact gtgcagatgc aactgggtgg  
 9961 tgttttgtct atcttgaaat ggctcagta tgttctccc tcttctctcc gtcctcttcc  
 10021 tattgtaaaa tctcttgatc caccagagag agctgtaaca tactttccag agaattcatg  
 10081 tgatgcccgt gctgccatct tgggcattca ggacactcaa ggcaagtggg tgagtgggat  
 10141 gtttgacaga gaaagctttg tggaaacgtt agaaggatgg gccaaaactg ttattacygg  
 10201 aaggggcaag ctgggtggga ttccagttgg tatcatagct gtggaaaccc agacagtgat  
 10261 gcaagtaac cctgctgacc ctggctcagct tgattctgcc gagcgtgtag tccctcaagc  
 10321 kggacagggt tggttcccag attcggccgc aaaaacrgcc caggcactgc tggatttcaa  
 10381 ccgtgaagag ctcccgttgt tcatacttgc taactggaga ggcttttctg gtgggcaaaag  
 10441 ggaatctgtt gaaggaatcc ttcaaggctgg tyctatgatt gttgagaatc tgaggacgta  
 10501 yaagcagcct gcttttgtgt acataccaaa ggctggagag ctgcgtggag gtgcatgggt  
 10561 tgtgtgggac agcaagatca atccggagca cattgagatg tatccgaga ggactgcgag  
 10621 aggggaatgc cttgagggac cgggactcat tgagatcaa ttcaagccaa atgaattgga  
 10681 agagagtatg ctagggtctg accctgagtt gatcagcctc aatgctaaac tccctcaaga  
 10741 aactagtgtc agccctagcc cttgggaaac ggcggcggcg gcagagacca tcaggaggag  
 10801 catggctgct cggaggagc agctgatgccc catataactc cagggttgcca ccgggtttgc  
 10861 tgagtgcac gacacctccg caagaatggc tcttctacag gagactgca aggaggcttg ccgaggactc  
 10921 ctgggaggag tcccgggcct tcttctacag gagactgca aggaggcttg ccgaggactc  
 10981 gctcgccaaa caagtcagag aagccgcccg cgagcagcag atgccactc acagatcagc  
 11041 cttggagtgc atcaggaaat ggtacctggc ctctcaaggg ggagacggcg agaagtgggg  
 11101 cgatgatgaa gccttcttca cctggaaaga tgatcctgac aagtatggca agtatcttga  
 11161 ggagctgaaa gccgagagag cgtctacact gctgtcgcat ctgctgaaa cctcgacgc  
 11221 caaggccttg cccaacggtc tctcgctcct cctcagcaaa gtaagtcttct tttgcttatt  
 11281 agtatttgtt tgttcttcta tacatttctc aataagtttc tttgcttct tcttttcttt  
 11341 gttcttgtat agtttctcta attaaattct tctgtccct aagttcatct cctgataca  
 11401 tacatttgtat tgattgtaca gatggatcct gcaaaagagg agcaggttat ggatggcctc  
 11461 aggcagcttc ttggttgatt actggccccc gccctttgat aacgcatcca ttcagccagc  
 11521 ataatcggc cttgcttgtt gccaccaagc aagtcctgtc tatggtgggc tgggtaccag  
 11581 tggaacaagc aaattttact tgcgtggcga gctacaggag ggggaggatt ttcagcggaa

FIG. 3-6

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11641 gaaactgaa acacattgtt tgcacatagg taggagggcat ctcatctcag gacaatcygt  
11701 atgtttattg tcattacaga taggtacaca caaagcatat gtatgctgga tagatatctg  
11761 gtgtgagttg ttgcaatgca agattcatca tcttaattta cgagatacga tgtgatgac  
11821 ggtcgatgtg gtagtgttag ttctctcagt ggcagggaat gccgagtttc cttacgctgc  
11881 agttatgtga tatgtaaacc ctgagaactt tggggtgata tgatggacgt tttatcagtt  
11941 tcatgagaaa tgaatttggg gccgaggccc cttacatcag ttttttttct tcta

(SEQ ID NO:30)

FIG. 3-7

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MVESDQINGTPNRMSSVDEFCKALGGDSPIHSLVANNGMAAVK  
FMRSIRTWALETFGNEKAILLVAMATPEDLRINA EHIRIADQFL  
EVPGGTNNNNYANVQLIVEIAERTRVSAVWPGWGHASENPEL PD  
ALMEKGII FLGPPSAAMGALGDKIGSSLIAQAAGVPTLPWSGSH  
VKVPQETCHSIPEEIIYKNACVSTTDEAVASCQVVGYPAMIKASW  
GGGGKGIRKVHNDDEVRALFKQVOGEVPGSPIFIMKVASQSRHL  
EVQLLCDKHGNVAALHSRDCSVQRRHQKIIIEGPITVAPPETIK  
ELEQAARLAKCVQYQGAATVEYLYSMETGEYYFLELNPRLOVE  
HPVTEWIAEINLPASQVVVGMIPLYNIPEIRRFYGIEHGGGYH  
AWKEISAVATKFDLDKAQSVKPKGHCVAVRVTSED PDDGFKPTS  
GRVEELNFKSKPNVWAYFSVKSGGAIHEFSDSQFGHVFAFGESR  
SLAIANMVLGLKEIQIRGEIRTNVDYTVDLLNAAEYRENKIHTG  
WLDRIAMRVRAERPPWYLSVVGALYEASSRSSSVVTDYVGYL  
SKGQIPPKHISLVNLTVTNLIDGSKYTIETVRGGPRSYKL RINE  
SEVEAEIHSRLRDGGLLMQLDGN SHVIYAETEAAGTRLLINGRTC  
LLQKEHDP SRLLDATPCKLLRFLVADGSHVADTPYAEVEVMKM  
CMPLLLPASGVIFVMPEGQAMQASDLIARLDLDDPSSVRRAP  
FHGTFPKLGPTAISGKVHQKFAASVNSAHMILAGYEHNINHVV  
QDLLNCLDSPELPFLQWQELMSVLATRLPKDLRNELDAKYKEYE  
LNADFRKSKDFPAKLLRGVIEANLAYCSEKDRVT SERLVEPLMS  
LVKSYEGGRESHARAVVKSLEFEEYLSVEELFSDDIQSDVIERLR  
LQHAKDLEKVVIYVFSHQGVKSKNKLILRLMEALVYPNPSAYRD  
QLIRFSALNHTAYSGLALKASQLEHTKLSELRTSIARSLSELE  
MFTEEGERISTPRRKMAINERMEDLV CAPVAVEDALVALFDHSD  
PTLQRRVVETIYIRRLYQHYLARGSVRMQWHRSGLI ALWEFSEEH  
IEQRNGQSASLLKPQVEDPIGRRWGVMVVIKSLQLLSTAIEAAL  
KETSHYGAGVGSVSNGNPINLNGSNMLHIALVGINNQMSTLQDS  
GDEDQAQERINKLSKILKDN TITSHLNGAGVRVVS CIIQRDEGR  
SPMRHSFKWSSDKLYEEDPMLRHVESPLSTFLELDKVNLEGYN  
DAKYTPSRDRQWHMYTLVKNNKDP RSNDQRMFLRTIVRQPSVTN  
GFLFGSIDNEVQASSSFTSNSILRSLMAALEEIELRAHSETGMS  
GHSHMYLCIMREQRLFDLIPSSRMTNEVGQDEKTACTLLKHMVM  
NIYEHVGVRMHRLSVCQWEVKLWLDCDQANGAWRVVVT SVTGN

FIG. 4-1



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TCTVDIYREVEDPNTHKLFYRSATPTAGPLHGIALHEPYKPLDA  
IDLKRAAARKNETTYCYDFPLAFETALKKSWESGISHVAESNEH  
NQRYAEVTELI FADSTGSWGTPLVPVERPPGSNNFVVAWNMKL  
STPEFPGGREIIVVANDVTFKAGSFGPREDAFFDAVTNLACERK  
IPLIYLSATAGARLGVAEEIKACFHVGSDDQSPERGFHYIYLT  
EQDYSRLSSSVIAHELKVPESGETRWVVDITIVGKEDGLGCENLH  
GSGAIASAYS KAYRETFTLTFTVGTGRAIGIGAYLARLGMRCIQRL  
DQPIILTGY SALNKLLGREVYSSQMLGGPKIMATNGVVHLTVS  
DDLEGVSAILKWLSYVPPYVGGPLPIVKS LDPPE RAVTYFPENS  
CDARAAICGIQDTQGKWL SGMFDRESFVETLEGWAKTVITGRAK  
LGGIPVGI IAVETETVMQVIPADPGQLDSAERVVPQAGQVWFPD  
SAAKTAQALLDFNREELPLFILANWRGFSGGQRDLFEGILQAGX  
MIVENLR TYKQPAFVYIPKAGELRGGAWVVVDSKINPEHIEMYA  
ERTARGNVLEAPGLIEIKFKPNELEESMLGLDPELISLNAKLLK  
ETSASPSPWETAAAAETIRRSMAARRKQLMPIYTQVATRF AELH  
DTSARMAAGVISKVVDWEESRAFFYRRLRRRLAEDSLAKQVRE  
AAGEQQMPTHR SALECIRKWYLASQGGDGEKWGDDEAFFTWKDD  
PDKYKGYLEELKAERASTLLSHLAETSDAKALPNGLSLLL SKMD  
PAKREQVMDGLRQLLG

(SEQ ID NO:31)

FIG. 4-2

GGATCCTCTTGAGCTTCTTCAGCAGAGATACAGTTGACATGGCCACGTGCAGTGGTGGCTGGCTTGCGGTAGAACA  
CTTTCCCTGTGGCTTGCCACGGCCAAACAGCTTTTCCAGATTGGTTGGGTGGTCTGGGACACCTCGCGCAGATA  
GTGGCCCGGTTCCCACATTTAAAGCAAGTCACCGAGCTGTTACGTGGAGGACATTTGTTGGTTGGACCAACCATAG  
GGCTTGGCTGGTGTAAACTGTTGGCGGGCAAGCGCTGAAAGGATGGCTCGGTGTGAACCTCGGTGGCAGGG  
CAGTGTAGGCACCCACACACCGGCTTCTGAGGACCAACCGGATGAGGAACCCATGTCAACGGCCATGCTTGCG  
TGTTGCTTCAATAACAGTCTGACCAAGCTCAGCATTGATGGCTTGTTAACAAGCTTCTGAAGAAGATGTGCACTCA  
TGCACCGGAGGTGCGCGGAAGCTCAGGCTAAGTCCCCTATGGAACCTTGCTTGCTTCTTGCTTCAGTAGAGA  
CTTCCCTCAGTTGCATATCGTGCAAGGTTACCGAACTCCCTACTGTAAAGCATCCAAGAAAGTCGACCTTGAAGTGA  
ACTGCAGAACTCCTCATGTTTACGGTCCATGAGACCTTCCGGAATGTGATGTTTCAACGAAAGCTTCGCTGAATTCA  
GCCAGGTAGTGACATGGCCCGCTGGCGCATAGCTCCATAGTTCTCCACCATTAGATGCTGGCGGGCCCTTCAAGAT  
GATATGCAGCAAGGTGACCTTATCAGCCTCAGCTACTAGCGCAGAATGCACTTGTGAGTAATACTGCGAAGCCCA  
GTATCGGCGTCGAGAGGCTCGACGGAGTGGTGGAAAGTGGTGGATGTAACTTGATGAATCACTGAGTGACACC  
AAGTTATTCTCTGATGGTGTCCATGTTTGTCTCGATGGCTCCAAACAACGGTTAGTCTCCGCTTGTGTTCTCT  
CGGCTTCAGCATAACTTCGCCACAGAGAGGGAGGTGAGGCAGGTTGTTTCCCCCAACTCTGCTGCCCTTCGGCCTG  
CTCTGGGGAGCAGGGTTGTTGGGTGTAAACCATCCTAGGAAAAAACAATAGTTTAGTCCAGGATGATAGG  
ATTCTGACATAGAACGAAGAAATGTAATGGATAACTTGGAAATGTAAGATGACCATCCGTATGACATGGTAGATACAG  
AAACTGCTTCTTTTATTCATCGTCTATACACCACTACAAGGTTTAGTACAGAAACAAACAAGTACTACTACGGT  
GAAAAGAGGATTACATCTCATCGGAGGCAATCCGAGTCCCTATACATTAATTTTCTACACTCCGGAAGCGGTAC  
AAGCTAAGTATATCCACGAGTCACGCAAGGACGGTGGATGATACAGCTTAGTAGATAGTATGATATACTACTA  
ACTCAGACAACTCCGTAGTAGTCTTTCATATAAGTCACCTCCATAGCCTGGAAGCTCAACGTGATCGTGATCCTTCT  
TTTTCGTTGCTGAGGGCTGTTGGAGGGATTAATCATTCGCTCCAGAACTGATGACATCGGTTATGCACGT  
CCTATTTAAATCAGACATGAGTGAATAAAGTATGATATGACGTTATGGCGCAACCGACAAACATGGGAACATGA  
CATGTTTCATCTCCCAACATACACGAAACCAAGAAACAAACACCCCGCACTACGATTGGAGATGTAGGCATCA  
AAGCGTCGAGACCTATGCCAAGCACACCATCCATCTGTGACCATTGAAGCACAACTATTTCATCTTCCACCAGCCCC  
GCCTCCATGAATGTGGACTAGAAATGTGAATGTGTACTGCGCGTGGCGTGTGTCGTTTGCTTCGGCGGAACAC  
CACCAGCCGGTACAGCAAGCAATTTGTGACCGTCAACTAAATTTGGAATCGTTGGCGCATTAATCATTGGAAATATG  
CATGCTCCGTTAAGGCAACGGAACAATTAGCTAGACAAACACCCATGATGCAATTAGCTAGACAAATTAGCTAGA  
CAACACACCCACGGAACAATTAGCACCGACCTACGGGACGCGCGGACGGTGACGGGACGTTGGACGAACCGCAGC  
GGAGACGCCACCGGAGCGGAGGACGCTGAGCACATCGAGTCGAGGCAGACACACCCCGGAGACACCGGATGCA  
ACGACGACCCATCCGTCCATCCGCCCGCCAAACCGGCGCATGCGGCCCAACTACCCGTCGTCGCCGTCATAGACC  
ACGCCCCCACTGCCCGCCCAACCCCAACTCTCCATGAATGACGCAATTCATCGCTCCAACCCACAA  
CGCAGAGCCCGCAGCACAGCGGCTCGGCGACGCGCGCATTTATACACGCAATTCATCTGGATCTCCACC  
TGCCCGCAGCACGGGTTTCTCTCTCTCCCGCGCGCATTCGCTCGAACGGCTTGGCGGCGGCTCCGGACGG

FIG. 5-1

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ACCCACGGTAAGCTCCCCCTGGCCCTTGCTATGCCCCCTGCTTCTGCACGCATCTTCCGATTTTCGCTGGAGCGCTCC  
GCCTCCGCCCTATGCGTGGGGCGATTGACTGGGCCGGAATTGCCATGGACTCGTACTGACCACTGATGTACTCGCT  
CGCTAGCCTCTCCGCCACGCCGCCCTCAAAATCGAGCGCGGTAGGCTGCCCTCCAGGCCCAATCCAAGCAGCGCA  
GCGAGGGCCTTCCTGCTGATTCTCTCTCAGCGCCAGGAGATCAGGGACCCAGATACCACTGCTAGCAGTCGACCC  
GTGCCGT'CGCCGGAATGCGGGTTCGCCCGCTGCGCAATACGTGAGCGGTGGTGGCGCGCGGACTGGCCCGG  
GTTTTGGGCACACTTGTGCTTACTTCTTCTGCTGAATGCCGGAATCAAGTCCATTTCCCTCTTTGCTCCTGCT  
TGGACTAACCCAGTCCCCTAGTGTGACTACAGCAATTTTTCGCGTATTTTAAATGTGATCTCTGGTCTTGTCTCT  
CTGGTTCTGCTGTTGTTGACTAGAAATCTGCACTCTCCCATGGCACTTTGCCGAGGAATTTCCCGATTTAGCT  
AGCCGTTAATTAGTGCCACCATGTTGTTGTTTCTGTAGTACCATTTTAGCATCTGGTACAGAAAAGGGCACACA  
CATGCCAAACCGAAAAGAAATATCCCACTGCTGCAATTTCTACGCTAATCGGACATATAATGATTGATGCGCTAACGG  
ACGGACTTGTCTTTTGCTTTTCCACGCGCTGAAGGTTGGAGGGGCAATA

(SEQ ID NO:32)

FIG. 5-2

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TACTCGCCGCGGCGTAGGCATGGGCGTATGCATCCTACTGTTTCTGTGGATCTACTCGCCGCGGCGAG  
CGGCGTAGGCATGGGCGTGTGGGCGCTGCAGCACGCTGACGACGCTGGACAGGACTCGAGCCCATGGACAA  
CGTCAGCGGCTGCAGGAGGCGACAGGCTTGGCGGTCAGCCCTTCGACGCTCTCGTCTCGTCTCG  
CCGCTGCAGCTCGTAGACGCTCTCTGCAACGCGCCATCATAGGCTCTGCGCCCATGCGCGCGGACAGTCG  
CCATGGCTTCCGCGCGTCCGCTCTCTGCACTAGTGTCTGCGCGCTGCGCCGCTGCGCCGCTCGGCTG  
AGTCGCGCCGCTGAGAGCCGCGCTGCTGTCAGTGTCTGCGCAACAGGACTGCCAGGCTGCTCGGATCGG  
CCGGACAGTTTCCACCCCTGATGATCAGGCGCGCTGCAATCAGACCGCGGCAACTCCGATGTCCAGACGGC  
GGGACAGAGGTGTGTAGCGTATCCTTGGCAGATGCAACGGCGGATAGTAAGAGGATAGAGAGATATGTT  
TTCAGCCGAGAAAGACAGGAAGGATGACGACGTAGATAGACGCGGAGGATGAAGGGCATGTTTGGAT  
GCCGATAGCATGAGATGCGGGCGGGAAGAGATCAATTAGGTTGAGTGGCTTCTATTTTAGCTGATAATAAAT  
TAGATGACAAATATATATGTTAGGATTAAGTTTAAATAGGATGATTTGCTGAGATTAGTTTCTTAATAG  
GATGATGCACCTGATTTAGTTTATAGAAAGGATGACCGGATATATATAGTTTCTTAATTGCCAGGCGTGG  
AGTTTCAATATTTCTCACAGTGGATACGGCAGTCAATGTAATTGCTAAGTGACACAGAAATGTTTAGG  
TTAAGGCTAACCGTTAGATTGATTTAGTGGCTTAATCGTGGTATGATTTGATCTGTGTACGCTTGTGGGT  
GTGCTTAAAAAGTTCTTAATTGATTTTAAATAGTAGTATAGATAAAAAAGGCACGCTTCTGTTAACGCGCGTAG  
AAAAATATTTGAATCACAAACAGAGCTAAACAAAGCATGATATGCCCTTGTGGCAAAACCGGTGACACGGGAGT  
ACAAATGTTTCCACCAACACGTCACCCGAGAAACGGAATAAACACCCCGCAGTATGTTTGGAGGCTTGGCATC  
AAAAGGTTGGACCTATGCTAGGCACAAATCCATCCGTGACGCGGAGCGCAACTATGCTTCAAGGGGAAAT  
GGAATCGACTCCGACCAACGGGAGCGGAGGAGTCTACATCACCCGTCAGTGTCCCGCCCGTAAATGCAC  
GACTAGAAGGTGCACATTTGCTCTCAAAAAGAAAAAGCAATCAACCTGTGTTGTTGTTGTTAGAGGG  
ACTGTGTTATCCCGAGCCCATGTTCAATCTGTTGCTCGCATTTATTTCTGGAATTTATTTAGGATTTCCG  
GCGATGCGCATTCAGTGGAGGTTTATAGGATGAGTGTATACCGGTGATATAGCGCTTGTGCTGTACTGTGT  
TAAAAAAGAAAAAAGATATGTACCATTCGCGGTATGTCCATACACTGAGCCGATTAGCTAGAGAAC  
AGGTCATGATGAGTCCGAGTTACGGTAAACGAAACGAGGAGTCAACAGGCGGCACAGACGCGTGGTGGCT  
TGGCCGACGACTACGGACGCGCGGACGCGTGGGACGTTGAGCGAAGCCGAGGAGCAACGCAACCGAGCGGAA  
GGAGCGAGCATCGAAGGCGTTGGGCTTACCTACACACGCGGAGAGACAGGTGCAACGACACCAATCC  
GTCCAACGAGGCGATGAGGCGCAACAACTGTGTCGACTCTCCCGCTCCACCTCCACCAACCGCCACCT  
GCCCCCGCCACCCCAACCCCACTCTCCATGAATGACGCAATTCATGCTCCTACCAACGACGAGCAG  
CACCAGCGGCTCGGCGACGCGCGGCAATTTATAGCAAGCAATTCCTGTTGCTCCGCTCCGCGCGCTGCC  
TCTCTGGATCTCCATCTGGCCGAGACGCGCTTCTTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT  
CGGCGCGGCTCGGCGCAACCGACGTTACGCGCTTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT  
CCCCCTCTTCCGTTTCTGCTGAGCACCGCTGCTGTGTAGTGTGTTAGCGAGTGGTCTCGCTACTGGCT  
TCGGCCGAGTGGCTGCTCCGCGCGCGGCTAAGAACAGTAGTACTACCAACGCTTCTCTCTCTCTCTCTCT  
CTTCAATCGAGCACGAGCCGCTAGCTCCAGGCCCCCAGTCCCGCAAGCGCGCGGCGCTTCTCTCTCTCTCT

FIG. 6-1

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AGCGCACGAGATCAGGAGCCGGATACTGCTCTCGCGCGCGGATTTCGAGCTAGTTCTGTCGCGCGGAGTCCTGC  
TGACGCGGATCCTGCCGACATCGACCCGCGCTCGCCGAATTGGCGGCGGCTTCTTCGTGCCGTCTGGCATT  
ACGTCGAGCGGGTGGCGTGCGTGATTTGGCGGGTTTGGGTGCTTGTCTTCCGTCTTGTGCTGAATGTC  
GGAATTCAAGTCCCTTTTCCCTTCGCTCCTGCTGGAGTGAACCTTAGTGGACTTCAACATTTTTCAT  
TGTGATCTAGGGTCTTGTCTGTTCTGCTGGCTGTGACTATCAGCTTACTGTTGCGGATTGCGCACTTCC  
CCTGGCACTGTTCCGGAGGAATTTCTGATTTTCTTATTAGTGGTTAAATAGTACCATTATGTCCTTTGTTT  
GCTTTGTGCCATTTTAGCATCCAGTACAGAAAAAGGAATAAACGTGCAAACTGAAAAATAATAACCCGGTGC  
TGTTTTTCGCTAACAGACAGAAATTGATTCACCATTTTCTGATTTAGTTAGTTAGTTAAATAGGACTACTATGTTT  
TTGTTCTGTTTGTACCATTTTAGCATCTAGTACAGAAAAAGCGCACACATGCCAAACCGAAAGAAATATCCCA  
ATGCTGCAATTTCTACGCTAATCGGACATAAATGATTGATGCGCTAACAGACGGATTGTTGTTCTTTTGTCTTTCCAG  
TGCTGAAGGTTGGAGGGGCAATA

(SEQ ID NO:33)

FIG. 6-2

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<b>(30) Priority Data:</b> 08/422,560 14 April 1995 (14.04.95) US 08/468,793 6 June 1995 (06.06.95) US 08/611,546 5 March 1996 (05.03.96) US		<b>(88) Date of publication of the international search report:</b> 01 May 1997 (01.05.97)	
<b>(71) Applicant:</b> ARCH DEVELOPMENT CORPORATION [US/US]; 1101 East 58th Street, Chicago, IL 60637 (US).			
<b>(72) Inventors:</b> HASELKORN, Robert; 5834 S. Stony Island Avenue, Chicago, IL 60637 (US). GORNICK, Piotr; Apartment 1705, 5050 S. Lake Shore Drive, Chicago, IL 60615 (US).			
<b>(74) Agent:</b> KITCHELL, Barbara, S.; Arnold, White & Durkee, P.O. Box 4433, Houston, TX 77210 (US).			
<b>(54) Title:</b> ACETYL-CoA CARBOXYLASE COMPOSITIONS AND METHODS OF USE			
<b>(57) Abstract</b> <p>The present invention provides isolated and purified polynucleotides that encode plant and cyanobacterial polypeptides that participate in the carboxylation of acetyl-CoA. Isolated cyanobacterial and plant polypeptides that catalyze acetyl-CoA carboxylation are also provided. Processes for altering acetyl-CoA carboxylation, increasing herbicide resistance of plants and identifying herbicide resistant variants of acetyl-CoA carboxylase are also provided.</p>			

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## INTERNATIONAL SEARCH REPORT

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PCT/US 96/05095A. CLASSIFICATION OF SUBJECT MATTER  
IPC 6 C12N15/52 C12N15/82 C12Q1/68 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X	PLANT MOLECULAR BIOLOGY, vol. 22, 1993, pages 547-542, XP002026093 GORNICKI, P., ET AL.: "Wheat acetyl-CoA carboxylase" see the whole document --- -/--	6-8,11

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

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Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Int'l Application No  
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